Evaluation natural preservatives of *nothopanax scutellarium* merr. leaf extract in physical characteristics of nile tilapia (*Orechromis niloticus*)

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**Abstract.** *Nothopanax sutellarium* is one plant that is known to be used as an antimicrobial because it has several active ingredients such as alkaloids, saponins, flavonoids, and polyphenols. This study aims to determine the effect of *Nothopanax sutellarium* leaf extract with different solvents, namely water, ethanol, and nanoemulsion solvents on the physical characteristics of tilapia. This type of research is experimental research with a quantitative approach. This research was conducted in Biology Education Laboratory Universitas Syiah Kuala, Chemistry Education Laboratory of Universitas Syiah Kuala, and Pharmacology Laboratory of Universitas Syiah Kuala. This research uses a completely randomized design (CRD) factorial pattern with Factor A (solvent extract) and Factor B (concentration) consisting of 6 treatments and 4 replications. Data were statistically analyzed using Analysis of variance (ANOVA) and BNJ (Honest Significant Difference) at a significant level of $\alpha=0.05$. Based on the results of research giving *Nothopanax sutellarium* leaf extract with different solvents significantly influence the shape of the eye, gill color, aroma, and meat texture in Nile tilapia. Treatment with extract concentration of 60% using nanoemulsion technique is the best treatment. *Nothopanax sutellarium* nano emulsion extract has the potential as a natural ingredient in preserving fresh fish during transportation.

1. Introduction
Nile tilapia is a freshwater fish with a food commodity that is widely cultivated in Indonesia and is in demand by the public because the meat is thick, dense, relatively cheap to consume, and has good nutritional value. Tilapia is a fish that is easily cultivated and developed in both the pond and cage area and tilapia is one of the sources of animal protein needed for humans [1]. Nile tilapia is the most popular freshwater species because of its compatibility with the various cultures of the world, containing essential nutrients, a delicious taste. neutral, has the ability to adapt to various conditions in the environment, and is a species capable of exploiting sources of nutrition from the lowest.

Nile tilapia have characteristics the lines (bars) found on the tail fin and dorsal fin [2]. Tilapia has five fins, namely dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin. Oreochromis mozambicus and Oreochromis niloticus are invasive species that are highly desirable in the world of freshwater cultivation because these two species are relatives of the Chichlid family which have a high tolerance level for various environmental conditions [3]. Tilapia is a perishable food that is caused by protein content and environmental factors that facilitate the growth of rotting microbes [4]. Tilapia are the most commercial freshwater species because of their high nutritional value, can grow and develop rapidly and are more resistant to pathogens [5]. Furthermore, Ariestya et, explained that fish are a lot of people
in the community because they have low economic value, are tasty, and have 43.76% protein chemistry, 7.01% fat, 6.80% gourd content, and 4.28% water per 100g of meat. Oreochromis niloticus is a potential export commodity in Indonesia [6].

Efforts can be made to extend the shelf life of Nile tilapia with natural preservatives derived from plants, namely various types of fruits and vegetables [7]. *Nothopanax sutellarium* is one of the plants which is known to be used as a natural preservative because it has antimicrobial compounds. *Nothopanax sutellarium* is an ornamental plant or hedge plant that has the characteristics of dark green leaves, thin, compound leaves, round, curved like a bowl, the base is in the shape of a heart, has jagged edges, and pinnate repetition [8]. *Nothopanax sutellarium* contain flavonoids, alkaloids, saponins, polyphenols, fat, calcium, phosphorus, iron, vitamins A, B, and C. Flavonoids, alkaloids, and saponins have the ability to be useful as antimicrobials. *Nothopanax sutellarium* is a plant that grows a lot in Indonesia, usually this plant grows in the yard of the community [9].

In this study, *Nothopanax sutellarium* leaf extract used three different solvents, namely water solvent, ethanol solvent, and nanoemulsion solvent. Nanoemulsion is a transparent or clear system with very uniform and very uniform globule sizes. Nanoemulsion has the advantage of a very small droplet size which can prevent flocculation, can pass through rough skin surfaces, can increase drug penetration, and has a transparent color that can provide aesthetic appearance when used [10]. This study aims to evaluation of *Nothopanax sutellarium* leaf extract with different solvents, namely water, ethanol, and nanoemulsion solvents on the sensory characteristics of Nile tilapia.

### 2. Methods

This research was conducted at Biology Laboratory FKIP in Universitas Syiah Kuala University, Chemistry Laboratory FKIP Universitas Syiah Kuala, Pharmacology Laboratory of the Faculty of Veterinary Medicine Universitas Syiah Kuala Syiah Kuala and PVKK Laboratory FKIP Universitas Syiah Kuala Syiah Kuala.

This research is an experimental study and a factorial completely randomized design (CRD) research method with 6 treatment dan four replication., with two factors, the first factor was the extract solvent, namely water, ethanol, and nanoemulsion solvents, while the second factor was the extract concentration.

- P0: Negative control (without extracting)
- P1: Positive control (iced)
- P2: 20% *Nothopanax sutellarium* leaf extract
- P3: 60% *Nothopanax sutellarium* leaf extract
- P4: 80% *Nothopanax sutellarium* leaf extract
- P5: 100% *Nothopanax sutellarium* leaf extract

#### 2.1. Procedure of making nothopanax sutellarium leaf extract

*Nothopanax sutellarium* leaves are washed, cut into small pieces, and dried in the room. The dried *Nothopanax sutellarium* leaves are crushed using a blender until they become powder and *Nothopanax sutellarium* leaves are ready to be macerated [11]. *Nothopanax sutellarium* leaf powder was macerated into aquadest solvent to extract with aquadest solvent for ± 72 hours and *Nothopanax sutellarium* leaf powder was macerated with 70% ethanol solvent to extract with ethanol solvent. After 24 hours, the *Nothopanax sutellarium* leaf powder soaked in ethanol is replaced with a new solvent so that all active substances are extracted completely. Solvent replacement is carried out 3 times. The solution obtained is then evaporated using a rotary evaporator. The preparation of *Nothopanax sutellarium* leaf nanoemulsion extract consists of an oil phase and a water phase. The oil phase formulation consisted of 60 ml of *Nothopanax sutellarium* leaf extract and the water phase consisting of 60 grams of malrodextrin, 6 ml of tween 80, and 34 ml of phosphate buffer solution. Homogenization of the oil phase and the water phase using a centrifuge for 20 minutes [12].

#### 2.2. Treatment of Nile tilapia physical characteristics

All equipment is sterilized using 70% alcohol and then dried. Then each plate was labeled and put fresh Nile tilapia and then given *Nothopanax sutellarium* leaf extract with water, ethanol, and nanoemulsion
solvents with a concentration of 20%, 60%, 80%, and 100%. The physical characteristics of the tilapia were observed by giving a score on each parameter which includes eye shape, gill color, meat texture, and fish aroma according to the guidelines on the assessment card for the physical characteristics of tilapia then each score is added and the results of the assessment are then analyzed. using Analysis of Variance. Time to observe the physical characteristics of tilapia was carried out every 4 hours for 32 hours.

2.3. Parameters of observed fish physical characteristics
Observation of the physical condition of tilapia was carried out every 4 hours for 32 hours of treatment. The parameters observed were physical conditions including eye shape, gill color, meat texture and aroma, which can be seen in Table 1.

Table 1. Physical characteristics parameters of fish observed.

| No. | Specifications                                                                 | Parameter Observed                        | Score |
|-----|---------------------------------------------------------------------------------|--------------------------------------------|-------|
| 1   | Eye shape                                                                       | Convex eye shape, cornea and pupil are clear, shiny. | 9     |
|     |                                                                                 | Flat, cornea and pupil clear, slightly shiny. | 8     |
|     |                                                                                 | Flat, slightly cloudy cornea, slightly grayish pupil, slightly shiny. | 7     |
|     |                                                                                 | Slightly concave, cornea slightly cloudy, pupil slightly grayish, slightly shiny | 6     |
|     |                                                                                 | Slightly concave, cornea cloudy, pupil slightly grayish, not shiny. | 5     |
|     |                                                                                 | Slightly sunken, cornea cloudy, pupils gray, not shiny. | 3     |
|     |                                                                                 | Very concave, cornea cloudy, pupil gray, not shiny. | 1     |
| 2   | Gills color                                                                     | dark red, brilliant with a hint of transparent mucus | 9     |
|     |                                                                                 | Dark red, less brilliant with a hint of transparent mucus | 8     |
|     |                                                                                 | Pink with a little bit of mucus. | 7     |
|     |                                                                                 | Pink with slightly cloudy mucus. | 6     |
|     |                                                                                 | Pale pink with cloudy mucus. | 5     |
|     |                                                                                 | Gray with clotted milky white mucus | 3     |
|     |                                                                                 | Gray with lumpy brown mucus. | 1     |
| 3   | Aroma                                                                           | Very fresh aroma, strong specific type | 9     |
|     |                                                                                 | Fresh, less specific types. | 7     |
|     |                                                                                 | Neutral. | 6     |
|     |                                                                                 | Slightly sour smell. | 5     |
|     |                                                                                 | Strong sour smell. | 3     |
|     |                                                                                 | Strong foul smell. | 1     |
| 4   | Texture meat                                                                    | Texture Dense, compact, very elastic | 9     |
|     |                                                                                 | Solid, compact, elastic. | 8     |
|     |                                                                                 | Slightly soft, slightly elastic. | 7     |
|     |                                                                                 | A little soft, a little less elastic. | 6     |
|     |                                                                                 | Slightly soft, less elastic. | 5     |
|     |                                                                                 | Soft. | 3     |
|     |                                                                                 | Very soft. | 1     |

(Source: SNI 2729, 2013)

2.4. Data analysis technique
Data were analyzed descriptively and quantitatively. The collected data will be tabulated in tabular form, this is done to make it easier in table experiments or facilitate the data analysis process. The percentage of freshness of fish was calculated using the formula P = f / n x 100%. The data on the effect of extracting and organoleptic tests obtained were analyzed statistically using ANOVA with SAS 9.3.1 Software.
3. Results and discussions
The results of the study on the effect of giving *Nothopanax sutellarium* leaf extract with different solvents, among others, measured the shape of the eyes, color of gills, aroma of fish, texture of meat, freshness of Nile tilapia.

3.1. Eye shape
The average physical characteristics of Nile tilapia Eye Shape after giving *Nothopanax sutellarium* leaf extract was the highest at a concentration of 60% (NP3) with an average of 7.24, while the lowest number was in treatment P0 (negative control) with an average of 5.06. The average value of the test results for the physical characteristics of the eye shape can be seen in Figure 1.

![Figure 1. Physical characteristics of nile tilapia eye shape.](image)

The results of the analysis of variance showed that immersion of Nile tilapia in mangkokan leaf extract had a significant effect on the eye shape of Nile tilapia. Based on the results of the analysis of variance on the shape of the eye, there is a significant difference at the 0.05. Based on Table 2 shows that treatment P0 (-) has the lowest score when compared to treatment P1 (+), P2, P3, P4, P5. The highest score was the P3 treatment, namely giving *Nothopanax sutellarium* leaf extract with nanoemulsion solvent to Nile tilapia.

| Treatment | Water | Ethanol | Nanoemulsion |
|-----------|-------|---------|--------------|
| P0        | 5.06  | 7       | 5.06         |
| P1        | 6.51  | 7.03    | 6.65         |
| P2        | 6.23  | 6.08    | 6.37         |
| P3        | 6.53  | 7.24    | 6.53         |
| P4        | 5.74  | 6.43    | 5.85         |
| P5        | 6.04  | 6.04    | 6.04         |

Table 2. The average physical characteristics of nile tilapia eye shape.

Treatment P0 (-) is Nile tilapia without treatment with the result that all of them have eyes that are not bright and sunken. In the P3 treatment Nile tilapia soaked with a concentration of 60% *Nothopanax sutellarium* leaf extract) is the best concentration in giving mangkokan leaf extract with a slightly convex eye condition. Eyes are the main indicator that consumers look at when buying fresh fish. Changes in the eye of Nile tilapia during storage are a characteristic of the deteriorating quality of the fish and unsafe for consumption. This is caused by the development of bacteria in the eye that becomes sunken and the rays fade [13]. The difference in value and quality in the appearance of the eye shape is
due to differences in the amount of substance concentration in *Nothopanax sutellarium* leaf extract so that it has a different effect [14]. The addition of mangkokan leaf extract can inhibit the growth of bacteria in the eyes of tilapia because *Nothopanax sutellarium* leaves contain active ingredients such as alkaloids, saponins, flavonoids, and folifenols [15].

### 3.2. Gills color

The average of physical characteristics of the color of the gills of Nile Tilapia after giving *Nothopanax sutellarium* leaf extract was the highest score at a concentration of 60% (NP3) with an average of 7.29, while the lowest score was in treatment P0 (negative control) with an average of 5.12. The physical characteristics of the color of the gills can be seen in Figure 2.

![Figure 2. Physical characteristics of Nile tilapia gill color.](image)

The results of the analysis of variance showed that giving of Nile tilapia in *Nothopanax sutellarium* leaf extract had a significant effect on the color of Nile tilapia gills. Based on the analysis of variance test results on the color of the gills there is a significant difference at the α=0.05. Based on Table 3, it shows that treatment P0 (-) has the lowest score when compared to treatment P1 (+), P2, P3, P4, P5. The highest score was in the P3 treatment, namely the provision of *Nothopanax sutellarium* leaf extract with nanoemulsion technique to Nile tilapia.

| Factor A (Extract) | Factor B (Treatment) | P0 | P1 | P2 | P3 | P4 | P5 |
|--------------------|----------------------|----|----|----|----|----|----|
| Water              | 5.12<sup>Aa</sup>   |    |    | 7.06<sup>Ae</sup> | 6.56<sup>Ad</sup> | 7.12<sup>Aef</sup> | 6.35<sup>Ac</sup> | 5.96<sup>Ab</sup> |
| Ethanol            | 5.12<sup>Aa</sup>   |    |    | 7.06<sup>Ae</sup> | 6.82<sup>Bd</sup> | 7.26<sup>BF</sup>  | 6.65<sup>Be</sup>  | 5.99<sup>Abb</sup> |
| Nanoemulsion       | 5.12<sup>Aa</sup>   |    |    | 7.06<sup>Ae</sup> | 6.4<sup>Ca</sup>   | 7.29<sup>Bc</sup>  | 6.77<sup>Cc</sup>  | 6.34<sup>Cb</sup> |

Based on the results of the test results, the color of the gills has a low value, namely the P0 (-) treatment, namely Nile tilapia fish without bulbs with the results that all of them have white gills and mucus. In the P3 treatment Nile tilapia soaked with a concentration of 60% *Nothopanax sutellarium* leaf extract is the best concentration in giving mangkokan leaf extract with the condition that the color of the tilapia gills has started to fade so that the color of the gills has turned pale red to slightly white. Fish gills are among the most susceptible to rot compared to other organs due to the accumulation of bacteria.
in high numbers in the gills. Gills are one of the parameters in this study because gills are a part of the fish body where bacteria are found and gills are also used as a consideration for consumers in choosing fresh fish when consumers want to buy fish for consumption [16]. The addition of natural ingredients containing antibacterial compounds can inhibit bacterial growth in the gills. Flavonoids in mangkokan leaves are compounds that function as antibacterial by forming complex compounds against extracellular proteins [17].

3.3. Fish aroma
The average physical characteristics of Nile tilapia aroma after giving Nothopanax sutellarium leaf extract was the highest at a concentration of 60% (NP3) with an average of 6.9, while the lowest score was in treatment P0 (negative control) with an average of 4.87. The average test results for the physical characteristics of tilapia aroma can be seen in Figure 3.

![Figure 3. Physical characteristics of nile tilapia aroma.](image)

The results showed that giving tilapia in Nothopanax sutellarium leaf extract had a significant effect on the aroma of tilapia (Table 4). Based on the results of the analysis of variance on fish aroma, there is a significant difference at the level of α = 0.05. Based on Table 4, it shows that treatment P0 (-) has the lowest score when compared to treatment P1 (+), P2, P3, P4, P5. The highest score in the P3 treatment was the provision of Nothopanax sutellarium leaf extract with nanoemulsion solvent to Nile tilapia.

| Faktor A (Extract) | Faktor B (Treatment) |
|--------------------|----------------------|
| Water              | P0 (-)  | P1 (+)  | P2     | P3     | P4     | P5     |
| Ethanol            | 4.87\(\text{ab}\) | 6.68\(\text{bc}\) | 6.24\(\text{bcd}\) | 6.76\(\text{A}\)  | 6.07\(\text{bc}\) | 5.55\(\text{Ab}\)  |
| Nanoemulsion       | 4.87\(\text{ab}\) | 6.68\(\text{bc}\) | 6.32\(\text{bcd}\) | 6.81\(\text{Abef}\) | 6.12\(\text{Abc}\) | 5.60\(\text{Ab}\) |

Based on the results of the aroma condition test that has a low value in the P0 (-) treatment Nile tilapia without clumping with the results all have a foul aroma. In the P3 treatment (giving Nile tilapia with a concentration of 60% Nothopanax sutellarium leaf extract) is the best concentration in giving Nothopanax sutellarium leaf extract with the condition that the tilapia smell is fishy. The occurrence of
foul odor in P0 (-) Nile tilapia is caused by the presence of microbes. The occurrence of fish rot is rancid. These changes occur due to the oxidation of fat causing unwanted rancidity. The changes that occur in fish odor are due to the breakdown of proteins from bacterial activity. One of the compounds, namely flavonoids, is a phenolic compound that gives plants red, blue, purple, and yellow colors [18]. Phenolic compounds function to form complex compounds with extracellular proteins, this can disrupt the integrity of the bacterial cell membrane [19].

3.4. Meat texture
The average texture of Nile tilapia meat after giving Notopanax sutellarium leaf extract is the highest at a concentration of 60% (NP3) with an average of 7.28, while the lowest score is in treatment P0 (negative control) with an average of 4.87. The average test results for the physical characteristics of Nile tilapia meat texture can be seen in Figure 4.

![Figure 4. Physical characteristics of tilapia meat texture.](image)

The results of the analysis of variance showed that giving of Nile tilapia in mangkokan leaf extract had a significant effect on the texture of tilapia meat. Based on Table 5 it shows that treatment P0 (-) has the lowest score when compared to treatment P1 (+), P2, P3, P4, P5. The highest score was the P3 treatment, namely giving Notopanax sutellarium leaf extract with nanoemulsion solvent to tilapia.

| Factor A (Extract) | P0  | P1  | P2  | P3  | P4  | P5  |
|-------------------|-----|-----|-----|-----|-----|-----|
| Water             | 4.87<sup>bc</sup> | 6.93<sup>bc</sup> | 6.55<sup>bd</sup> | 7.09<sup>bc</sup> | 6.31<sup>bc</sup> | 5.93<sup>bc</sup> |
| Ethanol           | 4.87<sup>bc</sup> | 6.93<sup>bc</sup> | 6.62<sup>ABd</sup> | 7.21<sup>bc</sup> | 6.51<sup>bc</sup> | 6.09<sup>bc</sup> |
| Nanoemulsion      | 4.87<sup>bc</sup> | 6.93<sup>bc</sup> | 6.77<sup>Ccd</sup> | 7.28<sup>C</sup> | 6.77<sup>C</sup> | 6.19<sup>bc</sup> |

Table 5. The average score of physical characteristics of tilapia meat texture.
Based on the results of the condition test for the texture of tilapia meat which has a low value, that is, in the P0 (-) treatment Nile tilapia without clumping with the results that all of them have very soft meat. In the P3 treatment (Nile tilapia soaked with a concentration of 60% Nothopanax sutellarium leaf extract) is the best concentration in giving Nothopanax sutellarium leaf extract with a slightly stiff meat texture and a little softer. This is because enzymatic processes begin to break down the muscle tissue of the flesh, the soft meat indicates complete rigormortis. Rigormortis changes are the result of a complex series of chemical changes in fish muscle after death [20, 21]. According to Wijaya et al (2018), mangkokan leaves have chemical compounds of flavonoids, saponins, and tannins. Saponins cause bacterial cell death by diffusing through the outer membrane so that the bacterial cell wall becomes easily damaged. Furthermore, saponins bind to the cell cytoplasmic membrane which will cause disruption of cell stability, as a result the cytoplasm leaks and leaves the cell. This resulted in the bacterial cell experiencing death. Tannins also have astringent compounds that can induce the formation of complex bonding compounds with enzymes or microbial substrates [19].

4. Conclusions
Giving Nothopanax sutellarium leaf extract with water, ethanol, and nanoemulsion solvents can effects the physical characteristics of Nile tilapia which include eye shape, gill color, aroma, and meat texture. Giving Nothopanax sutellarium leaf extract P3 treatment with a concentrazation of 60% using a nanoemulsion technique suitable for preservative in Nile tialpia. Administration of Nothopanax sutellarium leaf extract could be use preservative in fish fresh.

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