Effectiveness Test of Red Dragon Fruit Skin \textit{(Hylocereus costaricensis jack)} as Natural Preservation for Nila Fish \textit{(Oreochromis niloticus)}

T W Siregar\textsuperscript{1}, Z lubis\textsuperscript{2}, and E Julianti\textsuperscript{3}

\textsuperscript{1} Magister Student of Food Science, University of North Sumatera, Prof.A.Sofyan Number 3 USU Campus, Medan, Indonesia-20155.
\textsuperscript{2} Departmen of Food Science, University of North Sumatera, Prof.A.Sofyan Number 3 USU Campus, Medan, Indonesia-20155.
\textsuperscript{3} Department of Food Science, University of North Sumatera, Prof.A.Sofyan Number 3 USU Campus, Medan, Indonesia-20155.

Corresponding author’s email address: wardanisiregar05@gmail.com

Abstract. Indonesia is a tropical country, many plants suspected as potential antimicrobial so it can used as natural preservatives, one of all is red dragon fruit peel. Red dragon fruit (Hylocereus costaricensis jack) production now has been increasing. So, dragon fruit skin contributes as environmental trash so it must used as a natural preservative. The purpose of this study was to determine whether the red dragon fruit peel extract (Hylocereus costaricensis jack) used as a natural preservative in tilapia, to determine the characteristics and duration of tilapia storage after being given red dragon fruit peel extract consisting of three types of contractor (water, ethanol and acetate), after getting the best results, applied it to fish whether it affects the shelf life of tilapia (L\textsubscript{0}, L\textsubscript{1}, L\textsubscript{3}, L\textsubscript{5}). The research method used is a completely randomized design (CRD) with two factorial. The parameters tested were microbiology, proximate and organoleptic quality tests. The yield of 1% ethyl acetate is the best and the safest solution to be applied to tilapia. TPC in tilapia that have been preserved 1% dragon fruit peel extract is 3.53 \times 10^5 (L\textsubscript{0}) and 5.10x10^6 (L\textsubscript{3}). TVB levels in preserved tilapia were 67 mgN / 100g (L\textsubscript{0}) and 25.02 mgN / 100g (L\textsubscript{5}). Fish protein content from 18.5% (L\textsubscript{1}) to 15.10% (L\textsubscript{5}). Water content from L\textsubscript{1} 78.1% to L\textsubscript{3} 81%. Based on results it can conclude that red dragon fruit peel extract contains various bioactive compounds: alkaloids, flavonoids, glycosides, saponins and tannins. The best solvent for binding active compounds is ethanol but the best for food products is ethylacetate.

Keywords: Effectiveness Test; Red Dragon Fruit Skin, Natural Preservation, Nila Fish

1. Introduction

Dragon fruit plants growing and fruit fully with good quality when planted in the affluent state of the environment (climate and soil). Best temperature for dragon fruit is 22°C-35°C, the humidity is 40% - 60%, the altitude of lowlands to the medium is 0-500 m above sea level, the texture and structure is clay and sandy or gravel, soil contain excessive iron can interfere with plan growth that usually occurs in wet soils, good soil properties if the soil contains a lot of soil organic (humus) and soil organisms (soil microbes) decompose [1].
Red dragon fruit skin contains active compounds including alkaloids, terpenoids, flavonoids, tianine, niacin, pyridoxine, kobalamin, phenolic, carotene, and phytoalbumin, besides the superiority of dragon fruit skin is full of polyphenols and antioxidants, besides that Antioxidants in dragon fruit peels are higher than antioxidant in meat, so it has the potential to be developed into natural antioxidants [2]. Phenol and flavonoid organic compounds found in the phytochemical test of red dragon fruit peel extract. The results of the study [3] showed that extracts from red dragon fruit peel had a higher flavonoid content than the fruit, while the phenol content was lower. The phenol mechanism as antibacterial agent is poisoning the protoplasm, damaging and penetrating the walls and precipitating bacterial cell proteins. Compounds phenolic Large molecular are able to activate essential enzymes in bacterial cells even in very low concentrations. Phenols can damage the bacterial cells, denature proteins, activate enzymes and cause cell broken [4].

Tilapia generally live in fresh waters, such as rivers, lakes, reservoirs, swamps, rice fields and irrigation channels, but salinity tolerance of tilapia can live and breed in brackish waters with salinity that is preferred between 0-35%. Freshwater tilapia moved to brackish water, with a gradual adaptation process of small tilapia 2-5 cm, more resistant to environmental changes than older fish. Sudden removal can cause the fish to stress or even die [5].

2. Methods

2.1 Research design
This study uses 2 factors: the first factor is type of solvent (water, ethanol and ethylacetate) and the second factor is storage duration (1 day, 2 days, 3 days and 5 days). The best solvent will applied to tilapia, then stored until the fifth day and analyzed changes during storage.

2.2 Material and tools
Equipment consists of laminar flow, autoclaving, Rotary Evaporator, paper discs and Colony Counter. The materials used are red dragon fruit skins obtained in the North Sumatra region, tilapia fish obtained from fish ponds in the North Sumatra area. Chemicals and media of olivesutrient agar (NA), Mueller Hinton Agar (MHA), physiological NaCl 0.9%, ethylacetate, ethanol, water, bacterial culture of Staphylococcus aureus, Escherichiacoli and Lactobacillus acidophilus.

2.3 Observed parameters

2.3.1 Phytochemical Test of red dragon fruit peel extract Phytochemical
Testing of red dragon fruit peel extract is active compounds present in red dragon fruit peel extract, including testing of flavonoids, alkaloids, steroids and free form triterpenes, saponins, tannins and glycosides.

2.3.2 Inhibitory zone activity test
Testing of microbial activity in red dragon fruit peel extract using 6 mm diameter blank disc paper. Diluted extract solution. 100% concentration was obtained by adding 2g of red dragon fruit peel extract and added 2 ml of DMSO, from 100% the concentration was made. Then the disc paper immersed in the extract solution with various variations of the concentration for approximately one hour until the extract solution diffuses well into the disc paper. 10 ml of MHA media was poured into a sterile petri dish and allowed to condense. A sterile cotton swab dipped in a culture suspension is gently applied to the media surface, then dry at room temperature for several minutes. Discs containing extra red dragon fruit peels with concentration placed regularly on the surface of the test media using sterile tweezers. Furthermore, the microbial culture incubated for 24 hours at 37°C. After incubation, the diameter of the inhibition zone around the disc was then measured by calipers. Microbial inhibitory activity observed from the antimicrobial inhibition. Inhibitory zone area was formed as the parameter for selecting red dragon fruit peel extract used in the next step.

2.3.3 Determination of Minimum Inhibitory Concentration (MIC)
**Minimum Inhibitory Concentration** (MIC) is lowest concentration of antimicrobial compounds growth of bacteria. The tested bacteria used for the determination of the MIC antimicrobial substrate is *Escherichia coli*, *Staphylococcus aureus* and *Lactobacillus acidophilus*. MIC determination aims to get the minimum concentration of red dragon fruit peel extract which is able to inhibit the growth tested bacteria including pathogenic bacteria. MIC is the lowest extract concentration that can inhibit the growth of test bacteria in paper disc diffusion method with extract concentrations of 0, 0.25%, 0.50%, 0.75%, 1%, 2%, 3%, 4%, and 5%.

### 2.3.4 Testing total volatile base (TVB)
Sample fish that had been drained as much as 10 g was added to distilled water to 300 ml and added 2 mg of magnesium oxide, diwadah recipients included 25 ml of boric acid and three drops of indicator methyl red, do distillation for 25 minutes of distillation titrated with H$_2$SO$_4$ 0, 05 N. The TVB content is calculated using the following formula:

$$\text{Kadar TVB} = \frac{V \times N \times 103 \times 14}{W}$$  \hspace{1cm} (1)

where V is ml H$_2$SO$_4$, N is normality, and W is sample weight.

### 2.3.5 Fish sensory Characteristics
During saving fish 1, 2, 3 and 5 days, observations of fish sensory characters were carried out. Sensory observations of fish known through the color and fish color flesh appearance, aroma and texture.

### 3. Results and discussion

#### 3.1 Phytochemical test results of Red Dragon Fruit Skin Extract
Testing of red dragon fruit skin extracts was carried out with the aim of identifying the components of the bioactive compounds contained in the red dragon fruit peel extract. Phytochemical test results with three different types of solvents can be seen in Table 1 as follows:

| Bioaktive Compound     | Solvent Skin Extract Red Dragon Fruit |     |
|------------------------|--------------------------------------|-----|
|                        | Water      | Etanol | Etil | Asetat |
| Alkaloid               | -          | +      | +    |        |
| Flavonoid              | +          | +      | +    |        |
| Glikosida              | +          | +      | +    |        |
| Saponin                | -          | +      | +    |        |
| Tanin                  | +          | +      | +    |        |
| Tritterpen/steroid     | -          | +      | -    |        |
| Glikosida Antrakuinon  | -          | +      | -    |        |

From the phytochemical screening results, it is known that the alkaloid compound was obtained in the extract of the skin of red dragon fruit with ethanol and ethylacetate solvents. Flavonoid compounds, glycoses and saponins are found in water, ethanol and ethylacetate solvents. Whereas saponin compounds found only in water and ethanol, while for triterpenes or steroids found only in ethylacetate solvents. We found ethanol as the solvent that contains the most bioactive compounds. Ethanol and ethylacetate are organic solvents, so alkaloids can attracted to these solvents. Akaloid is a polar compound. In its free form, alkaloids are weak bases that are difficult to dissolve in water but easily dissolve in organic solvents [6].

#### 3.2 Antibacterial Activity of Red Dragon Fruit Skin Extract Against Escherichia coli, Staphylococcus aureus, and Lactobacillus acidophilus
Based on the results of antibacterial activity tests on red dragon fruit peel extract against bacteria *Escherichia coli*, *Staphilococcus aureus* and *Lactobacillus acidophilus* by means of inhibition zone testing on these bacteria, it can be produced as follows:

![Table 2](image)

| Bacteria                  | Solvent | Zone Diameter (mm) |
|---------------------------|---------|--------------------|
| *Eschericia coli*         | Water   | 9.05               |
|                           | Etanol  | 13.03              |
|                           | Etilasetat | 14.06          |
| *Staphilococcus aureus*   | Water   | 7.09               |
|                           | Etanol  | 13.05              |
|                           | Etilasetat | 14.08          |
| *Lactobacillus acidophilus* | Water   | -                 |
|                           | Etanol  | -                 |
|                           | Etilasetat | -            |

Based on table 2 on antimicrobial tests results of dragon fruit peel extract with three types of solvents, we can conclude that the three types of solvents produce inhibitory zones against the bacteria *Escherichia coli*, *Staphilococcus aureus* and not to *Lactobacillus acidophilus* bacteria. Antimicrobials test material called strong inhibitors when they have inhibition zones greater than 11 mm, moderate inhibits with inhibition zones 6-11 mm, whereas the inhibition zones are smaller than 6 mm that is inhibitory weak [7].

### 3.3 Minimum Inhibitor Concentration Test (MIC)

![Table 3](image)

| Extract Concentration (%) | *Staphylococcus aureus* | *Escherichia coli* | *Lactobacillus acidophilus* |
|---------------------------|-------------------------|-------------------|-----------------------------|
| 5                         | +                       | +                 | -                           |
| 4                         | +                       | +                 | -                           |
| 3                         | +                       | +                 | -                           |
| 2                         | +                       | +                 | -                           |
| 1                         | +                       | +                 | -                           |
| 0.5                       | -                       | +                 | -                           |
| 0.25                      | -                       | -                 | -                           |
| 0.1                       | -                       | -                 | -                           |

Information: (+) The extract make influence to microbial activity  
(-) The extract cannot affect to microbial activity

MIC test in this study carried out on *Escherichia coli* bacteria, *Staphilococcus aureus*, not to *Lactobacillus acidophilus*. The bacterium used which is thought to grow a lot in freshwater fish species. Table 3. Shows that the MIC value in red dragon fruit peel extract is about 1%. Furthermore, it used for application of red dragon fruit peel extract on fresh tilapia fillets. In table 3. above, it can conclude that red dragon fruit peel extract affect the microbes growth, namely red dragon fruit peel extract from 5% to 0.1% For *E. coli* and *Staphilococcus aureus* microbes while formicrobes *Lactobacillus dysentria* there’s nothing affect for all concentration.

### 3.4 TPC (Total Plate Count)

On TPC analysis, the number of bacteria on tilapia analyzed (0 days) was $3.70 \times 10^5$. Table 5 shows that tilapia that were not added to red dragon fruit peel extract on the second, third and fifth days experienced an increase in TPC very significant, namely on the second day $3.26 \times 10^6$ to $7.20 \times 10^6$ on the fifth day. Standard fresh fish quality according to BPPOM TPC maximum limit is about $5 \times 10^5$ so
that tilapia without adding red dragon fruit peel extract is not suitable for consumption on the third day. Tilapia with red dragon fruit peel extract only slightly changed the TPC value from the first day to the third day that can consumed, but on the fifth day, tilapia can't consume because it exceeds the TPC standard value. This is because the red dragon fruit peel extract contains antimicrobial compounds and antioxidants such as flavonoids, saponins, and tannins that capable in inhibiting microbes growth for tilapia. Other chemical components that become the main chelating spasmodic effect is can reduce cell membrane so it can interfere with cell permeability. Because of the disruption of permeability, cells cannot carry out living activities so can effect to their growth and could die [8]. This can causes antimicrobial effects by extract of red dragon fruit peel or skin in inhibiting growth of microbes in tilapia fillets.

3.5 Analysis Total Volatil Base (TVB)
Benefits of measuring TVB levels are to find out the freshness level in fish, as well as a measuring tool for consumption. Fish have been decayed if they have a TVB level > 30 mgN / 100g [10].

![Figure 1. Comparison of TVB levels of tilapia treated with red dragon fruit peel extract compared with untreated tilapia stored for 5 days and fresh tilapia as a control](image)

Fish contains several enzymes that can reduce protein. The enzymes found in digestive organs are trypsin, kemotripsin, pepsin. Therefore, the decomposition process discharged from the stomach is decaying process more slowly than fish that in line with stomach inside. The accumulation of volatile nitrogen compounds in fish save in a cool ice occurs more slowly than fish stored at normal temperature [9].

3.6 Quality of Organoleptik Test
Organoleptic testing conducted to determine the level of tilapia panelist acceptance. Sensory parameters for tilapia are color, smell and aroma. Effect of red dragon fruit peel extract on the color, smell and texture of tilapia can known in Figure 2.
During storage affects, changes of texture determined by the water, carbohydrate and protein. The longer immersion or storage is carried out, the more active components can maintain the texture of the fish [10].

4. Conclusions
Compounds that play an important role in the preservation process (antimicrobial) in red dragon fruit peel extract are alkaloids, flavonoids, glycosides, saponins and tannins and the best type of solvent for binding active compounds present in the skin of red dragon fruit is ethanol, but the best inhibition that is collaborate of red dragon fruit peel extract and ethylacetate.

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