Addition Of Flax Seed Oil *(Linum usitatissimum)* As A Source Of Omega-3 In Snakehead Fish *(Channa striata)* Albumin Ointment On Closure Of Wound Healing

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Abstract- Ointment is a semi-solid preparation intended for topical application to the skin or mucous membranes. Usually snakehead fish albumin is consumed in extract form so that people do not like it because of its smell. One alternative to using albumin, which is easy to apply to help the wound closure process, is to use it as an ointment. The advantages of the ointment are good for the drug delivery system, pleasant in appearance and comfortable feeling after use. The results of stage 1 research showed that differences in the concentration of flaxseed oil affected the quality of albumin ointment, that is pH, viscosity, omega-3, protein content, fat content, water content, fatty acid profile, and zinc. The best quality of albumin ointment was obtained at a flaxseed oil is 2.5% with the following results pH 6.7, viscosity 18,960 cP, omega 3 at 18.43%, protein content 1.94%, fat content 74.86%, and 10.98% water content. Then proceed to animal trials of mice on the 3rd, 5th, and 7th day of the observation showing that the best snakehead fish albumin ointment treatment experienced the fastest wound closure process with a wound closure character of 75% on the 7th day.

Index Terms- ointment, albumin, wound closure, fatty acid

I. INTRODUCTION

Snakehead fish is rich in nutrients needed by the body, especially protein. Albumin is a globular protein that is often applied clinically for nutritional improvement and postoperative wound closure. Albumin functions to regulate the osmotic pressure in the blood, maintain the presence of water in the blood plasma so that it can maintain blood volume in the body and also as a means of transport or transportation (Firlianty et al., 2019). Apart from albumin, an important component in the wound closure process is also influenced by collagen, omega-3 and omega-6 fatty acids (Nicodemus et al., 2014). The collagen used is collagen from grouper skin because grouper fish has the best collagen content (Suprayitno, 2019). Flaxseed oil is a source of omega-3 which has a high α-linolenic acid level of 57%. A-linolenic acid is a polyunsaturated fatty acid that cannot be synthesized by humans (Putri and Ardiaria, 2016). The source of omega 6 fatty acid is obtained from sunflower seed oil.

Usually snakehead fish albumin is consumed in extract form so that most people do not like it because of its smell. One alternative to using albumin, which is easy to apply to help the wound closure process, is to use it as an ointment. Ointments are semi-solid preparations intended for topical application to the skin or mucous membranes (Yamlean et al., 2019). The advantages of ointment are good for the drug delivery system, pleasant in appearance and comfortable feeling after use (Tungadi et al., 2011). This study was used to develop an albumin extract with the addition of collagen, a fatty acid from essential oils containing omega 3 and omega 6 to form an ointment so that people can easily apply it to cuts.

II. MATERIALS AND METHODS

2.1 Materials

The research materials that used consist of raw materials for making albumin extract, that is snakehead fish, materials for the manufacture of collagen, namley grouper fish skin, NaOH, acetic acid and distilled water. The ingredients for making albumin ointment are albumin extract from snakehead fish extraction, flaxseed oil as a source of omega 3, collagen, sunflower oil as a source

of omega 6, and fillers in the form of adeps lanae, vaseline flavum, butylated hydroxytoluene (BHT) propylene glycol (PG) , methyl paraben and propyl paraben.

This research method is an experimental method carried out in 2 stages, stage 1 and stage 2. Phase 1 research aims to obtain the optimal concentration of flaxseed oil added to the production of albumin ointment in order to have the best ointment quality for research in stage 2. While the second stage study aims to determine the wound closure process in experimental animals using the best quality albumin ointment compared to positive control and negative control.

Research 1 aims to obtain the optimal concentration of flaxseed oil added to the production of albumin ointment in order to obtain the best ointment quality for the second stage study. While the second stage study aims to determine the wound closure process in experimental animals using the best quality albumin ointment compared to positive control and negative control.

2.1.1 Albumin Extractiom

The production of albumin extract begins with the preparation of raw materials. The fresh snakehead fish is turned off and weeded, then filled, cut into cubes and then washed. Furthermore, the snakehead fish is weighed 250 grams, then put into the steamer pan. The snakehead fish meat is steamed for 30 minutes at 70oC. After that it is filtered and the filtrate is taken. Snakehead fish extract is ready to use.

2.1.2 Collagen Manufacture

The first step to make collagen is to prepare the raw material, namely the skin of the grouper fish which has been removed from the scales. Furthermore, the skin of the grouper is cut into 1 x 1 cm size. After that the grouper skin was soaked in 0.1 M NaOH for 24 hours. The ratio of skin and NaOH solution is 1:10. Then the grouper skin was neutralized with distilled water. After that the grouper skin was soaked in acetic acid for 24 hours with a ratio of skin and a solution of 1:10. Then filtered and taken the filtrate. After that, the precipitation was carried out for 24 hours with 0.9 M NaCl. Then it was centrifuged for 20 minutes at a speed of 3500 rpm. Then dialysis with 0.5 M acetic acid with a ratio of 1:10. Then inserted into the cellophane membrane. Then soaked in 0.1 M acetic acid solution for 6 hours. After that it is soaked in distilled water to neutral pH. Grouper skin collagen is ready to use.

2.1.3 Albumin Ointment Making

The process of making albumin ointment begins with preparing 3 beaker glasses. In the first beaker glass, put BHT and then crush it finely. Furthermore, the albumin extract is added. Then add a little adeps lanae and stir until homogeneous. In the second glass case, methyl paraben and propyl paraben were dissolved with propylenglycol. Then add a little adeps lanae and stir until homogeneous. In the third beaker glass, the two ingredients of the first beaker glass are mixed. After that, flaxseed oil was added with the respective concentrations of 0.5%, 1.5% and 2.5%. Then added collagen and sunflower seed oil. Then added to 20 grams of vaseline flavum. Albumin ointment was analyzed physically (pH and viscosity), chemistry (protein, fat, and water), and organoleptic.

III. RESULT AND DISCUSSION

3.1 pH

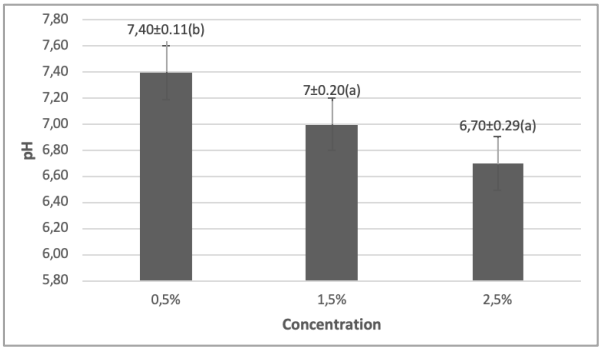

Figure 1. The Result of pH

The pH of the albumin ointment with different concentrations of flaxseed oil resulted in different pH values. At a concentration of 0.5% flaxseed oil, the resulting pH value was 7.4%, a concentration of 1.5% was 7, and a concentration of 2.5% flaxseed oil obtained a pH of 6.7. The highest pH value was obtained from the concentration of flaxseed oil 0.5% with a pH value of 7.4, and the lowest was obtained from a 2.5% flaxseed oil concentration of 6.7. Topical preparations are expected to have a pH that is at normal skin pH, namely 4.5 to 7 because if the pH is too alkaline it will result in scaly skin, whereas if the skin is too acidic it can trigger skin irritation (Patimasari et al., 2015).

3.2 Viscosity

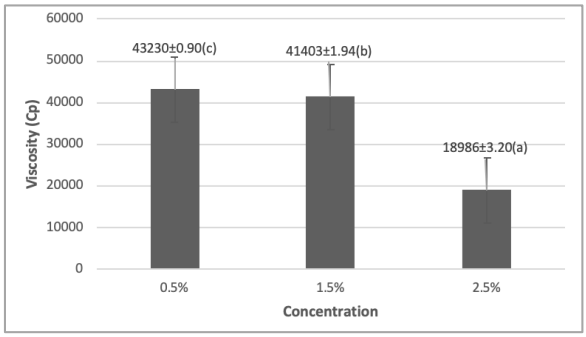

Figure 2. The Result of Viscosity

The viscosity value at 0.5% flaxseed oil concentration obtained a viscosity value of 43230 cP, a concentration of 1.5% of 41403 cP and a concentration of 2.5% of 18986 cP. The result of the viscosity of the ointment that had the highest value was albumin ointment with a flaxseed oil concentration of 0.5% as much as 43230 cP, and the lowest yield was at a concentration of 2.5% of 18986. Musdalipah, 2018).

3.3 Omega-3 Content

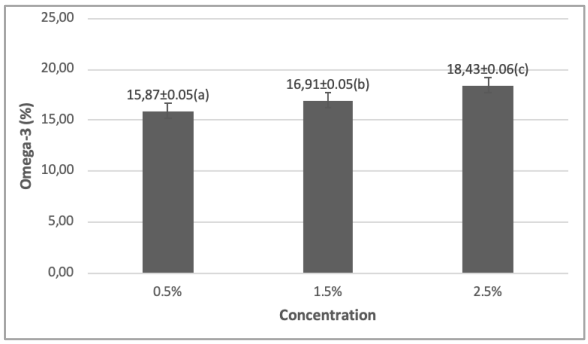

Figure 3. The Result of Albumin Content

The omega-3 content at the flaxseed oil concentration of 0.5% was 15.87%, the 1.5% concentration was 16.91%, and the omega-3 content of the flaxseed oil concentration 2.5% was 18.43%. The highest omega-3 levels were shown at a concentration of 2.5%, namely 18.430%, while the lowest levels of albumin were at a concentration of 0.5% at 15.87%. Omega-3 levels in the resulting albumin ointment showed an increase in line with the increasing concentration of flaxseed oil given.

3.4 Protein Content

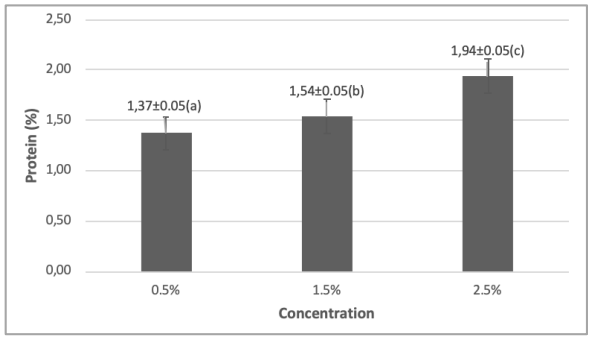

Figure 4. The Result of Protein Content

The protein content of albumin ointment at the flaxseed oil concentration of 0.5% was 1.37%, the concentration of 1.5% was 1.54%, and the concentration of 2.5% had a protein content of 1.94%. The highest protein content was obtained by hemp seed oil concentration of 2.5% at 1.94% and the lowest at a concentration of 0.5% at 1.37%. It can be concluded that the higher the concentration of flaxseed oil, the greater the protein content produced.

3.5 Fat Content

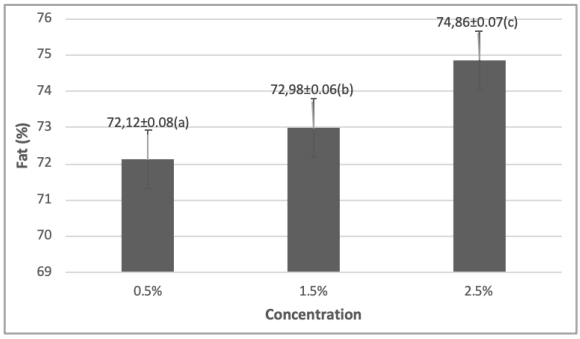

Figure 5. The Result of Fat Content

The fat content in the flaxseed oil concentration of 0.5% was 72.12%, the concentration of 1.5% was 72.98%, and the fat content in the concentration of 2.5% was 74.86%. The highest fat content was found in albumin ointment with a 2.5% concentration of 74.86%, while the lowest was a 0.5% concentration of 72.12%. The fat content in albumin ointment comes from the added omega 3 and omega 6 source oils, as well as the base of the ointment used.

3.6 Water Content

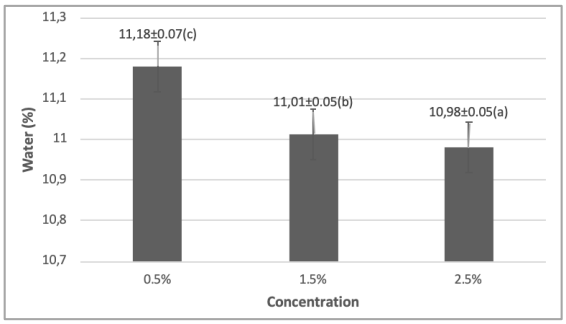

Figure 6. The Result of Water Content

The result of water content at 0.5% flaxseed oil concentration was 11.18%, at a concentration of 1.5% at 11.01%, and a concentration of 2.5% at 10.98%. The highest water content value was at a concentration of 0.5% at 11.18% while the lowest water content was at a concentration of 2.5% which was 10.98%. The value of the water content was getting lower along with the increase in the concentration of flaxseed oil applied to the albumin ointment.

3.7 Organoleptic

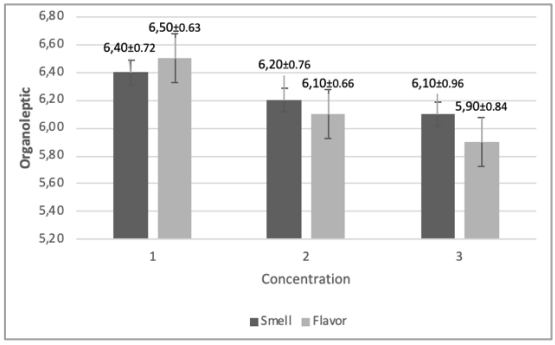

Figure 7. The result of Organoleptic

Organoleptic assessment of albumin ointment with different concentrations of flaxseed oil showed differences. In the treatment of flaxseed oil concentration of 0.5% the color parameter shows a value of 6.5, at a concentration of 1.5% of 6.1, and at a concentration of 2.5% indicates a value of 5.9. Meanwhile, the treatment of flaxseed oil concentrations with different aroma parameters showed different values. The aroma value of 0.5% concentration is 6.4, 1.5% concentration is 6.2, and at a concentration of 2.5% indicates a value of 6.1. The color and aroma parameters obtained the highest value respectively at the concentration of 0.5%, while the concentration that had the lowest value was the concentration of 2.5% flaxseed oil. The highest values for color and aroma parameters at a concentration of 0.5% were 6.5 and 6.4, respectively. The highest value indicated that the panelists liked the color and aroma of the albumin ointment which was produced from the 0.5% concentration of flaxseed oil. Meanwhile, the lowest value of color

and aroma parameters at 2.5% concentration treatment were 5.9 and 6.1, respectively. The lowest value obtained indicated that the panelists did not like the color and aroma of the albumin ointment resulting from a concentration of 2.5%.

3.8 Experimental Animal Testing

After knowing the optimal concentration of flaxseed oil for making the best quality albumin ointment, namely 2.5% concentration, then tested on experimental mice to determine the effect of albumin ointment on the closure of the cut, further tests were analysis of the fatty acid profile of albumin ointment, and zinc levels test. Albumin ointment with the best concentration of 2.5% was compared with negative controls and positive controls. Mice were injured 2 cm in length. The wound closure process was measured for 7 days with observations on the 3rd, 5th and 7th day.

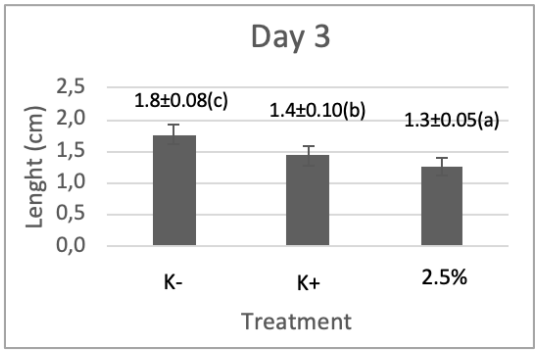

Figure 8. The Result of Day 3

On day 3, different treatments showed different results of wound closure. The negative control treatment showed an average wound length of 1.8 cm, the positive control treatment was 1.4 cm, and the albumin ointment treatment concentration of 2.5% was 1.3 cm. The best wound closure on day 3 was obtained by treatment of 2.5% albumin ointment with a remaining wound of 1.3 cm, while the longest wound closure was in the negative control treatment of 1.8 cm. This means that the wound closure process in the best treatment on day 3 was 35%.

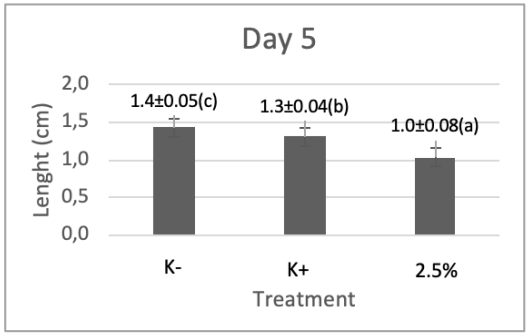

Figure 9. The Result of Day 5

Different treatments showed different results of wound closure on day 5. The negative control treatment showed an average wound length of 1.4 cm, the positive control treatment was 1.3 cm, and the 2.5% concentration of albumin ointment treatment was 1 cm. The best wound closure on the 5th day was obtained by the 2.5% concentration of albumin ointment, namely 1 cm, while the longest wound closure was in the negative control treatment of 1.4 cm. This means that the wound closure process on the best treatment on day 5 is 50%.

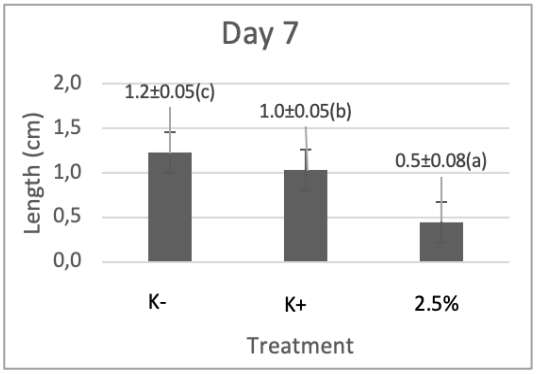

Figure 10. The Result of Day 7

The different treatments showed different wound closure results on day 7. Negative control treatment showed an average wound length of 1.2 cm, positive control treatment was 1 cm, and albumin ointment treatment concentration of 2.5% was 0.5 cm. The fastest wound closure on the 7th day was obtained by the 2.5% concentration of albumin ointment treatment, namely 0.5 cm, while the longest wound closure was in the negative control treatment of 1.2 cm. This means that the wound closure process on the best treatment on the day 7 is 75%.

3.9 Fatty Acid Profile

Jenis Asam Lemak	Unit	Result
Mono Unsaturated Fatty Acids (MUFA)		
Omega 9		
C 18: 1 (oleic acid)	%	15.04
Total	**%**	**15.04**
C 14: 1 (miristoleic acid)	%	3.09
C 16: 1 (palmitoleic acid)	%	4.34
Total	%	**22.47**
Poly Unsaturated Fatty Acids (PUFA)		
Omega 3		
C 20: 5 (eicosapentanoic acid)	%	4.39
C 18: 3 (linolenic acid)	%	12.90
C 22: 6 (decosahexaenoic acid)	%	1.14
Total	%	**18.43**
Omega 6		
C 18: 2 (linoleic acid)	%	16.54
C 20: 3 (eicosatrienoic acid)	%	3.74
Total	%	**20.28**
Saturated Fatty Acid (SFA)		
C 18: 0 (stearic acid)	%	1.98
C 16: 0 (palmitic acid)	%	6.02
C 12: 0 (lauric acid)	%	0.45
Total	%	**8.45**

The fatty acid content of albumin ointment had a different composition in the 20 grams of albumin ointment tested. The highest fatty acid content was found in omega 6 fatty acids, namely 20.28%, namely liloleic acid at 16.54%. While the lowest fatty acid content was obtained by saturated fatty acids at 8.45%, namely lauric acid at 0.45%. High levels of omega 6 are obtained from other components added to albumin ointment such as sunflower seed oil and snakehead fish albumin. The high and low levels of fatty acids in albumin ointment can be affected by the addition of other substances and storage temperature. If the storage temperature is higher, the fatty acid content will be lower.

3.10 Zinc Content

The best concentration of Zn in albumin ointment was 3.04 mg / 100g. Components that accelerate wound closure are the mineral zinc (Zn), omega 3 and omega 6 fatty acids in albumin ointment. Omega-3 fatty acids, especially EPA (Eicosapentaenoic acid) in albumin ointment and mineral content act as nutrients to accelerate incision wound closure by helping fibroblasts in synthesizing collagen to form scar tissue in incisions in the proliferation phase.

IV CONCLUSION

Flaxseed oil with a concentration of 2.5% is the optimal concentration to produce the best albumin ointment with a pH value of 6.7, a viscosity value of 18986 cP, an omega-3 level of 18.43, a protein content of 1.94, a fat content of 74.86, and a moisture content of 10.98.

The results showed that giving different treatments to wounds had a significant effect on the wound closure process. On the 3rd, 5th, and 7th day of observation, the best wound closure process was 75% in experimental animals treated with albumin ointment on day 7.

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