Accelerated Stability Test of Snakehead Fish Extract (Channa striata) and Kelulut Honey (Heterotrigona itama) Ointment Combination with Tween 80 and Span 80 as an Emulsifying Agent

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ABSTRACT

The combination of Snakehead Fish and Kelulut Honey has activity in accelerating the wound healing process. The ointment with active ingredients of Snakehead Fish extract and Kelulut Honey showed separation from the active ingredient and its base. The addition of tween 80 and span 80 is expected to increase the stability of the ointment preparation by keeping the water-phase droplets distributed uniformly on the ointment base. This study aimed to determine the effect of adding Tween 80 and Span 80 on the stability of the ointment preparation. The ointment was made by varying Tween 80 and span 80 by 2.5%, 5%, 7.5%, and control (active ingredients without the addition of tween 80 and Span 80) as a comparison. The preparation was subjected to an accelerated stability test using a temperature of 40 °C ± 2 °C / Relative humidity 75% ± 5% for 28 days by observing the organoleptic test, adhesion, spreadability, homogeneity test, pH test, and protection power. The data obtained were analyzed statistically using One Way Anova. The analysis showed that the addition of tween 80 and span 80 with a total concentration of 5% and 7.5% had a significant effect in increasing the spreadability, decreasing adhesion, and reducing the pH of the preparation. Formulas that meet the requirements in this study are F2 and F3, which have an average spreadability of 5.11 cm and 5.25 cm, adhesion of 96 seconds and 51 seconds, and pH of 6.02 and 5.02.

Keywords: Ointment; snakehead fish; kelulut honey; tween 80; span 80

INTRODUCTION

An ointment is a dosage form for external use that has an oil base, contrast to cream. The base is usually anhydrous (does not contain water), that almost all ointment preparations cannot be mixed well with skin secretions (Wijonarko, 2016). Ointment preparation is a semisolid preparation often used for wound healing (Wulandari et al., 2019). This study looked at the impact of including tween 80 and span 80 on the combination treatment of snakehead fish extract (Channa striata) and kelulut honey (Heterotrigona itama) as wound recuperating.

Snakehead fish is employed within the health sector as medicinal food because of its ability to accelerate healing wounds after surgery and childbirth. Snakehead fish contains high levels of amino acids and fatty acids and has a good capability in wound healing operations. Snakehead fish meat extract contains amino acids and fatty acids, substantial in the synthesis of collagen fibers, especially glycine, during wound healing. (Alviodinasyari et al., 2019).

Honey has had a special place in traditional medicine for centuries. The results showed that it is proven to contain antimicrobial substances which are active against the attack of various pathogenic germs that cause disease (Dewi et al., 2017). Kelulut honey has a distinctive taste, which tastes very sour. Generally, microorganisms cannot thrive in it because of the low water content and high acidity (pH) ranging from 3.2 to 4.5 to prevent the growth of microorganisms (Karnia et al., 2019).

Previous research combined snakehead fish extract and kelulut honey using adeps lanae as a base. Based on the orientation of the formula, there is a separation between snakehead fish extract and kelulut honey based on adeps lanae, so that in this research, the researcher took the initiative to add tween 80 along with span 80 as emulgators. Tween 80 and span 80 are non-toxic and non-irritating surfactants. Tween 80 and span 80 have a working system that acts as an emulsifier and conserves balance between hydrophobic and hydrophilic groups. Span 80 is an oil-soluble surfactant with a hydrophiliclipophilic adjust (HLB) of 4.3 and is hydrophobic, whereas Tween 80 may be a water-soluble surfactant and encompasses a hydrophiliclipophilic adjust (HLB) 15 which is hydrophilic.

Based on the description above, the researcher is interested in researching the effect of adding tween 80 and span 80 on the...
Accelerated Stability Test of Snakehead Fish Extract (Channa striata)

Table I. Ointment formula

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snakehead fish extract</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Kelulut honey</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Tween 80</td>
<td>-</td>
<td>0,865%</td>
<td>1,728%</td>
<td>2,592%</td>
</tr>
<tr>
<td>Span 80</td>
<td>-</td>
<td>1,635%</td>
<td>3,272%</td>
<td>4,908%</td>
</tr>
<tr>
<td>CMC-Na</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>0,18%</td>
<td>0,18%</td>
<td>0,18%</td>
<td>0,18%</td>
</tr>
<tr>
<td>Propylparaben</td>
<td>0,02%</td>
<td>0,02%</td>
<td>0,02%</td>
<td>0,02%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1,6%</td>
<td>1,6%</td>
<td>1,6%</td>
<td>1,6%</td>
</tr>
<tr>
<td>Adips lanae</td>
<td>Ad100%</td>
<td>Ad100%</td>
<td>Ad100%</td>
<td>Ad100%</td>
</tr>
</tbody>
</table>

Description: F0 = without tween 80 and span 80; F1 = Tween 80 and span 80 (2,5%); F2 = Tween 80 and span 80 (5%); F3 = Tween 80 and span 80 (7,5%)

...been heated and then expanded until the color is clear and homogeneous. Then add tween 80 and crushed until homogeneous (Mix 1). Adips lanae is crushed slowly until the color turns yellowish-white. Then, add span 80 and crushed until homogeneous. Next, add methylparaben and propylparaben that have been dissolved with propylene glycol, and crushed until homogeneous. (Mixed 2). then, add mixture 1 to mixture 2 and mixed until homogeneous.

Organoleptic tests
Organoleptic tests on preparations are carried out using the five senses to describe the shape or consistency (example: solid, viscous, liquid), color (example yellow or brown), and odor (aromatic or non-aromatic). The Organoleptic examination that includes texture, color, and odor is observed visually (Sandi & Musfirah, 2018).

Homogeneity Test
0.5 g of treatment was put on the object-glass equitably and outwardly watched. A homogeneous treatment is characterized by the nonappearance of lumps, as the result of basting (Sandi & Musfirah, 2018).

Spreadability Test
0.5 grams of treatment was set within the center of a circular glass. On high of it was put another circular glass and cleared out for a diminutive. Then, the diameter of the ointment that spreads was measured with no load and load weighing 50 g, 100 g, 150 g on days 0 to 28, measured using a caliper from various sides and stand for a minute then measured again the diameter that spreads from different sides, the dispersion test requirements for topical preparations are about 5-7 cm (Sandi & Musfirah, 2018).
Adhesion Test

0.25 g of the ointment is placed on a glass object whose area has been determined. At that point another glass object is set on beat. The glass object is connected to the test instrument and given a stack of 1 kg for 5 minutes. At that point discharged with 80 grams weight. Note the time until the glass objects are discharged (Sari et al., 2015).

Power Protection Test

The protection power test was carried out to see the capacity to ensure the skin from outside impacts, clean, and light at the time of treatment, which was demonstrated by no ruddy stains shaping after the expansion of KOH. The protection power was done by cutting filter paper (10 cm x 10 cm), then wetted it with a solution of PP (Phenolphthalein) as an indicator and dried then smeared, with an ointment preparation. Another filter paper (2.5 cm x 2.5 cm) is moistened with Paraffin Liquidum on the edges and waited to dry so that an area of the filter paper is bounded with Paraffin Liquidum. Filter paper smeared with ointment is affixed under the filter paper bordered with Paraffin Liquidum. The area part is moistened with KOH (0.1 N) solution. Observations were made at 15, 30, 45, 60 seconds, 3, and 5 minutes (Susilowati & Wahyuningsih, 2014).

PH test

The pH value of the ointment preparation was measured using a pH meter. PH measurement is done by dipping the pH meter into the ointment preparation. The preparation is dissolved first by dissolving 0.5 g of ointment in 5 mL of distilled water in a beaker. The pH meter is calibrated first using a standard buffer solution, 9. The electrodes are immersed in a beaker for 10 minutes. The pH value is viewed on a scale in the instrument and recorded after the pH value stabilizes. A good pH test of the ointment is 4.5-6.5 according to the pH value of human skin (Sandi & Musfirah, 2018) (Zulfa & Prasetyo, 2015).

Data Analysis

The research information were analyzed factually utilizing One Way ANOVA assisted by the SPSS program version 21, then continued with the Post Hoc test.

RESULT AND DISCUSSION

Organoleptic Test

The test results carried out at 40°C ± 2°C/RH75% ± 5% for 28 days did not show any changes in the ointment of snakehead fish extract and kelulut honey. The form and consistency of the control formula of ointment or (F0) from day 1 to day 28 have a denser consistency. This is because the formula with CMC-Na will form a hydrogen bridge that absorbs the water phase so that the consistency of the preparation tends to increase. The formula with the expansion of tween 80 and span 80 tends to be soft because tween 80 and span 80 causes the consistency of the dosage to decrease. Tween 80 and span 80 work by binding water molecules that cause the distribution of water molecules evenly on their bases, and the base used is an absorption base, namely adeps lanae. Adeps lanae can absorb the air and makes many air molecules scattered on the base of the adeps lanae, then the consistency of the preparation tends to decrease (Sandi & Musfirah, 2018) (Ayu et al., 2019). The color produced in the ointment preparation of snakehead fish extract and kelulut honey tends to be yellowish-white. This is because the honey kelulut and snakehead fish extract as active components in the preparation are yellowish-white. In addition, adeps lanae can also cause the color of the ointment to become slightly yellowish (Sandi & Musfirah, 2018). Observation of the odor of the ointment of snakehead fish extract and kelulut honey in all formulas resulted in a characteristic odor of kelulut honey.

Homogeneity Test

The test results carried out at 40°C ± 2°C/RH75% ± 5% for 28 days showed that the preparation formula without the combination of Tween 80 and Span 80 was not homogeneous on day 21 and day 28. This is indicated by the presence of lumps when the ointment is applied to the object-glass. While the ointment preparation with a combination of Tween 80 and Span 80 remained homogeneous until the 28th day. This means that tween 80 and span 80 has the effect of increasing homogeneity compared to ointments without tween 80 and span 80. The homogeneity of this ointment is influenced by the emulgitator. The addition of emulgitators in the form of nonionic surfactants, Tween 80 and Span 80 was dispersed on the base of the preparation so the formula with Tween 80 and span 80 became homogeneous. This is according to the requirements of the topical ointments that must show a homogeneous composition and do not show the coarse grain and uniform color (Tsabithah et al., 2020). Based on the above description, the F1, F2, and F3 formulas meet the homogeneity test requirements marked by the absence of lumps on the preparation and uniform color.
Spreadability test

The spreadability test was carried out for 28 days starting from the 1st day to the 28th day. This aims to see the effect of storage at a temperature of 40 ± 2°C with a humidity of 75% on the widespread of the ointment preparation. The results of the spreadability in each formula, F0, F1, F2, and F3 from day 1 to day 28 indicate an increase in the spreadability, this is indicated by the One Way ANOVA analysis and followed by the Post Hoc test that shows that all ointment formulas have a significant increase in the spreadability of the storage time on days 1, 3, 7, 14, 21, and 28 with conditions of temperature 40 ± 2°C and humidity of 75%. High temperatures can cause the consistency of the preparation to even be runner. The decreasing consistency of the preparation will increase the spreadability of the ointment preparation.

The control formula (F0), and the first formula (F1) have a spreading power of less than 5 cm. This is because the formula has a denser consistency. The addition of CMC-Na will form a hydrogen bridge that absorbs the water phase, so the consistency of the dosage tends to increase. (Forestryana et al., 2020). The second formula (F2) and the third formula (F3) meet the dispersion test requirements because they have a slightly soft or lower consistency. Tween 80 and span 80 work by binding water molecules that causes the distribution of water molecules on their bases. Adeps lanae can absorb water and makes many water molecules distributed on the base of the adeps lanae, so the consistency of the preparation tends to decrease and will cause the spreadability to increase (Ayu et al., 2019) (Sandi & Musfirah, 2018).

The preparations were compared between formulas on all test days (1, 3, 7, 14, 21, and 28 days) with the parametric test analysis, One Way ANOVA, then followed by the Post Hoc Test comparison test. This analysis was carried out to see whether the addition of tween 80 and span 80 had a significant increase in the spreadability of the ointment preparation or not. The results of the analysis showed that F0 and F1 did not have a significant difference, this means that Tween 80 and Span 80 with a concentration of 2.5% did not have a significant effect in increasing the spreadability of the preparation. The preparations of the F1 and F2 formulas have a significant difference. This means that Tween 80 and Span 80 with a concentration of 5% (F2) caused a significant increase in spreadability compared to a concentration of 2.5%. The ointment preparation between F2 and F3 was not significantly different, this means that Tween 80 and Span 80 with a concentration of 7.5% did not have a significant difference in increasing the spreadability compared to the addition of Tween 80 and span 80 with a concentration of 5%.

Based on the description above, the ointments that meet the results of the spreadability test are ointments with the F2 and F3 formulas, because, in these formulas, the addition of Tween 80 and Span 80 has a significant increasing the spreadability within the range of good dispersibility requirements 5-7 cm.

Adhesion test

Tests were carried out from day 0 to day 28 at extreme temperatures, namely 40°C ± 2°C and RH 75 ± 5% to observe the effect of the length of storage time on the adhesion of the ointment preparations. The results of adhesion in each formula, F0, F1, F2, and F3 from day 1 to day 28 show a decrease adhesion, this is confirmed by One Way ANOVA analysis and by a Post Hoc test which shows all ointment formulas have decreased adhesion was significant from the storage time. High temperature and humidity have an effect on decreasing adhesion. This can be related to the consistency that the thicker the ointment preparation will take, the longer it will

Table II. Spreadability test results (n=3)

<table>
<thead>
<tr>
<th>Day</th>
<th>F0±SD</th>
<th>F1±SD</th>
<th>F2±SD</th>
<th>F3±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.22±0.05</td>
<td>4.40±0.05</td>
<td>5.11±0.07</td>
<td>5.25±0.04</td>
</tr>
<tr>
<td>2</td>
<td>4.41±0.02</td>
<td>4.54±0.04</td>
<td>5.25±0.04</td>
<td>5.47±0.03</td>
</tr>
<tr>
<td>3</td>
<td>4.55±0.03</td>
<td>4.66±0.01</td>
<td>5.44±0.04</td>
<td>5.53±0.03</td>
</tr>
<tr>
<td>7</td>
<td>4.68±0.04</td>
<td>4.81±0.08</td>
<td>5.55±0.05</td>
<td>5.66±0.03</td>
</tr>
<tr>
<td>14</td>
<td>4.83±0.05</td>
<td>5.01±0.05</td>
<td>5.65±0.04</td>
<td>5.80±0.02</td>
</tr>
<tr>
<td>21</td>
<td>4.95±0.04</td>
<td>5.26±0.05</td>
<td>5.83±0.03</td>
<td>5.94±0.05</td>
</tr>
<tr>
<td>28</td>
<td>4.22±0.05</td>
<td>4.40±0.05</td>
<td>5.11±0.07</td>
<td>5.25±0.04</td>
</tr>
</tbody>
</table>

Description: F0 = without tween 80 and span 80; F1 = Tween 80 and span 80 (2.5%); F2 = Tween 80 and span 80 (5%); F3 = Tween 80 and span 80 (7.5%)
take to separate the two glass objects. High temperatures can cause the preparation to even be runnier. The decreasing consistency of the preparation will make the stickiness of the preparation decrease. All tested preparations met the criteria for good ointment adhesion, which is not less than 4 seconds.

The formula for the ointment preparation without tween 80 and span 80, control (F0), has a longer adhesion than the formula with tween 80 and span 80 (F1, F2, and F3). The addition of CMC-Na will form a hydrogen bridge that absorbs the water phase so that the consistency of the dosage tends to increase (Forestryana et al., 2020). Formula F1, F2, and F3 have less adhesion. This could be due to the addition of tween 80 and span 80 to reduce the stickiness of the ointment preparations. It is in line with other research which states that the combination of Tween 80 and Span 80 can reduce the adhesion of ointment preparations. In addition, tween 80 and span 80 work by binding water molecules that cause the distribution of water molecules evenly on their bases, besides the base used is an absorption base, its adeps lanae. Adeps lanae can absorb water, which makes many water molecules distributed on the base of the adeps lanae and the consistency of the preparation tends to decrease and will cause the adhesion to decrease (Ayu et al., 2019) (Sandi & Musfira, 2018).

The preparations were compared between formulas on all test days (1, 3, 7, 14, 21, and 28 days) with the parametric test analysis, One Way ANOVA and the Post Hoc Test comparison test. This analysis was conducted to see whether the addition of tween 80 and span 80 had a significant effect on reducing the adhesion of the ointment preparation. The results of the analysis showed that F0 and F1 did not have a significant difference, this means that tween 80 and span 80 with a concentration of 2.5% did not have a significant effect in reducing the adhesion of the preparations. The preparation between the F1 and F2 formulas also did not have a significant difference in reducing adhesion, while the F3 formula had a significant difference compared to F2. This means that tween 80 and span 80 with a concentration of 7.5% has a significant effect in reducing the adhesion of the ointment preparation.

Based on the description above, all ointment preparations meet the requirements for good adhesion, which is not less than 4 seconds. However, the addition of tween 80 and span 80 with a concentration of 7.5% has a significant effect in reducing the adhesion of the ointment preparation compared to the addition of tween 80 and span 80 with a concentration of 5%.

**Protection power test**

The ointment of snakehead fish extract and kelulut honey in all formulas could provide a protective power to the skin. This is indicated by the absence of red stains showing up on the filter paper dripped with 0.1 N KOH liquid, so the ointment meets the protective power standard of topical preparations.

**Ph test**

Ointments with the addition of tween 80 and span 80 tended to decrease the pH value. This could be because Tween 80 had a low pH, namely 2 (Rowe et al., 2009). The pH test results for 28 days of storage had a pH range of 4.96-6.36 and still within the physiological pH range of the skin 4.5-6.5 (Hasrawati et al., 2019). The pH test analysis was carried out using the parametric test, One Way ANOVA, then continued with the Post Hoc Test comparison. Ointment preparation was compared to pH on days 1, 3, 7, 14, 21, and 28 for each formula. Based on the One Way ANOVA test followed by the Post Hoc Test, it can be concluded that each pH of the 1st, 3rd, 7th, 14th, 21st, and 28th days experienced a significant difference. This

<table>
<thead>
<tr>
<th>Day</th>
<th>F0±SD</th>
<th>F1±SD</th>
<th>F2±SD</th>
<th>F3±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>124±4,51</td>
<td>97±2,51</td>
<td>96±2,64</td>
<td>51±3,78</td>
</tr>
<tr>
<td>2</td>
<td>85±4,50</td>
<td>88±2,00</td>
<td>70±4,04</td>
<td>43±6,81</td>
</tr>
<tr>
<td>3</td>
<td>80±2,08</td>
<td>81±3,05</td>
<td>67±5,03</td>
<td>38±3,05</td>
</tr>
<tr>
<td>7</td>
<td>65±3,53</td>
<td>73±2,52</td>
<td>61±5,51</td>
<td>31±2,64</td>
</tr>
<tr>
<td>14</td>
<td>56±2,08</td>
<td>65±2,64</td>
<td>46±2,54</td>
<td>26±3,05</td>
</tr>
<tr>
<td>21</td>
<td>46±3,60</td>
<td>30±4,58</td>
<td>22±1,52</td>
<td>8±2,08</td>
</tr>
<tr>
<td>28</td>
<td>124±4,51</td>
<td>97±2,51</td>
<td>96±2,64</td>
<td>51±3,78</td>
</tr>
</tbody>
</table>

Description: F0 = without tween 80 and span 80; F1 = Tween 80 and span 80 (2.5%); F2 = Tween 80 and span 80 (5%); F3 = Tween 80 and span 80 (7.5%)
Effect of Tween 80 and Span 80 on the pH Value of Ointment Preparation

The results of the analysis also show that F0, F1, F2, and F3 have a significant difference in the pH value. This means that the addition of Tween 80 and Span 80, with a total concentration of 2.5%, 5%, and 7.5% tends to lower the pH of the ointment preparation. This can be due to the influence of Tween 80 which has a low pH of 2 so that the increasing composition of the Tween causes the pH of the preparation to decrease. Based on the description above, all the preparation formulas, namely F0, F1, F2, and F3 meet the pH test requirements and the addition of Tween 80 and Span 80 causes a significant decrease in the pH value of the ointment preparation.

**CONCLUSION**

Ointment combination of snakehead fish extract and kelulut honey fulfills the stability requirements, which comprehend the organoleptic test, homogeneity test, pH test, adhesion, spreadability, and protective power. However, F1 and F0 do not meet the requirements for the dispersion stability test, and the control formula F0 on day 21 does not meet the condition of the homogeneity test. The formula that meets the stability requirements of a good ointment preparation in this study is a formula with the addition of Tween 80 and span 80 with a high total concentration of 5% and 7.5%.

**REFERENCES**


