Formulation and evaluation of the cream made from potassium azeloyl diglycinate as an anti-aging

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Abstract: Potassium Azeloyl Diglycinate has the ability to moisturize the skin, regenerate cells, increase skin elasticity, and hide fine lines. This research aims to discover the stability and effectiveness of anti-aging cream made from Potassium Azeloyl Diglycinate in regenerating and hydrating skin. The anti-aging cream formulation was made by varying the concentration of Potassium Azeloyl Diglycinate (0%, 3%, 5%, 7%) and then the physical properties stability was evaluated. The best formula was going to be tested for its ability to regenerate the mouse’s skin cells that had erythema, and hydrated the respondent's skin. In addition, an antioxidant activity was tested on pure Potassium Azeloyl Diglycinate and Vitamin C as the positive control. The results showed that the F2 Cream had the best physical properties stability evaluation results. The F2 Cream had the better ability to regenerate the mouse’s skin cells than the positive and negative control groups (the erythema diameter was 4.33±4.04 mm), and was also able to increase the skin moisture of 12 respondents who participated in the research. The results of the antioxidant activity test showed that Potassium Azeloyl Diglycinate belonged to the very weak antioxidant group (IC50: 83.946.587 ppm), while Vitamin C belonged to the very strong antioxidant group (IC50: 1.684 ppm).

1. Introduction
Premature aging is a process that is progressive and causing the skin structure and function to decline, caused by a decrease in the elasticity and collagen amount. This process will cause physiological changes such as dry skin, rough skin, uneven skin tone, enlarged pores, fine lines or wrinkles, and black spots [1]. Some other extrinsic factors that can trigger aging on skin are the heat influence, sleep position, lifestyle, and pollution[2].

In the skin conditions experiencing premature aging, we need a product whose functions are to protect and to improve the skin structure. Anti-aging creams function to stimulate the regeneration of damaged skin cells, maintain skin moisture, stimulate collagen production to increase skin elasticity, and become a source of antioxidants for skin tissue. The use of anti-aging cream is expected to slow down the process and reduce the signs of premature aging on skin. Potassium Azeloyl Diglycinate is a product of the reaction between chloride ion from azelaic acid compound with two molecules of glycine and KOH [3]. The research[4] on five respondents stated that Potassium Azeloyl Diglycinate was able to reduce excess oil production by 31.5%; increased stratum corneum moisture by 12.7%; and increase skin elasticity by 2.5%. The use of Potassium Azeloyl Diglycinate for 21 days has also been proven to reduce wrinkles on forehead by 11.7%, and on cheeks by 9.4%. Some other studies say
that Potassium Azeloyl Diglycinate is able to regenerate inflammatory and hyper-pigmented skin cells[5]. Potassium Azeloyl Diglycinate is more recommended to apply in an aqueous system formulation (the oil-typed cream in water (O/W), gel, and facial cleanser) with the concentration range of 3 to 10%[6]. This is what underlies the use of Potassium Azeloyl Diglycinate as a potential and stable active substance in the formulation of anti-aging cream type O/W.

Potassium Azeloyl Diglycinate has not been widely used in the cosmetic formulations in Indonesia until now, therefore, an anti-aging cream with O/W type formulation, containing active ingredient Potassium Azeloyl Diglycinate, is made. Furthermore, there will be an evaluation for the physical properties and stability test of each anti-aging cream formulation. The Potassium Azeloyl Diglycinate anti-aging cream with the best physical properties and stability will be tested for its effectiveness in regenerating the experimental animals’ skin cells with erythema, and hydrating the respondent's dry skin. An antioxidant activity test is also carried out on Potassium Azeloyl Diglycinate because there have not been any research that tested the antioxidant activity of Potassium Azeloyl Diglycinate. Eventually, the formulation of azeloglicina anti-aging cream will have the best effectiveness, safety, and acceptability.

2. Experimental
2.1. Materials
2.1.1. Instruments
0.5-g digital balance (Precisa XB 620C), digital balance (KERN ABJ, USA), porcelain cups (Pyrex), beaker (Pyrex), pipette dropper (Pyrex), stirring rod (Pyrex), water bath (Grant), volumetric flask (Pyrex), falcon, micropipette (DLAB), object glass, petri dish, weights balance, adhesive measuring device, stopwatch, pH meter (Ohaus Starter 300), viscometer (Rion Viscotester VT-04), spectrophotometer UV-Vis (Genesys™ 10S), vortex (Thermo), refrigerator, incubator (Memmert), skin moisture and oil content analyzer (SK-8), digital microscope (RoHS), and UV B 150 lamp (Exoterra).

2.1.2. Ingredients
Potassium Azeloyl Diglycinate (Innospec Active Chemicals), vaselinum album (white vaseline), mineral oil, liquid paraffin, stearic acid, glycerin, Nipasol, TEA, PGA, Nipagin, vanilla essence, Aqua Dest (distilled water), pH 7 phosphate buffer, 70% alcohol, product X (Unilever), DPPH indicator (HiMedia), pro analysis Vitamin C, pro analysis methanol (Merck), parchment paper, and blue tip (Nesco).

2.1.3. Experimental Animals
3-month-old male Wistar white rats, weighing 175 to 200 gram.

2.2. Methods
2.2.1. Making of Anti-Aging Cream
The anti-aging cream produced has the type of oil-in-water. The oil phase consists of vaselinum album, mineral oil, liquid paraffin, stearic acid, glycerin, and Nipasol. First, the vaselinum album and stearic acid were merged separately on a water bath, then mixed and added with other ingredients. The mixture was heated on the water bath to 70°C, and stirred until homogeneous. The water phase consists of TEA, PGA, Nipagin, Potassium Azeloyl Diglycinate, and Aqua Dest. First, the PGA was risen inside the Aqua Dest which had been heated previously on the water bath, then stirred until homogeneous, and added with other ingredients. The mixture was heated on the water bath to 70°C, then removed. The water phase was added little by little to the oil phase, and stirred manually and constantly in a counter-clockwise direction. The vanilla essence was added to the mixture and then stirred again until the temperature reached 35°C and formed a cream with thick consistency and homogeneous. It is modification of Dipahayu[7].
Table 1. Formulation of Potassium Azeloyl Diglycinate anti-aging cream with concentrations of 0%, 3%, 5%, and 7% in 100 gram cream

| Materials                  | F0  | F1  | F2  | F3  |
|----------------------------|-----|-----|-----|-----|
| Potassium Azeloyl Diglycinate | -   | 3   | 5   | 7   |
| Vaselin album              | 6.2 | 6.2 | 6.2 | 6.2 |
| Mineral oil                | 13.8| 13.8| 13.8| 1.8 |
| Liquid parafin             | 1.5 | 1.5 | 1.5 | 1.5 |
| Stearic acid               | 7.5 | 7.5 | 7.5 | 7.5 |
| Glyserin                   | 2.5 | 2.5 | 2.5 | 2.5 |
| Nipasol                    | 0.05| 0.05| 0.05| 0.05|
| TEA                        | 0.2 | 0.2 | 0.2 | 0.2 |
| PGA                        | 0.2 | 0.2 | 0.2 | 0.2 |
| Nipagin                    | 0.1 | 0.1 | 0.1 | 0.1 |
| Vanilla essence            | q.s.| q.s.| q.s.| q.s.|
| Aquadest                   | 67.95| 64.95| 62.95| 60.95|

2.2.2 Anti-Aging Cream Physical Properties Evaluation

2.2.2.1. Organoleptic Test
Organoleptic test included evaluation of changes in color, odor, consistency, and phase separation that are observed visually.

2.2.2.2. Homogeneity Test
A thin layer of cream was applied to a clean and dry object glass, then covered with another object glass, and observed for the homogeneity.

2.2.2.3. Viscosity Test
The viscosity test was carried out using a Rion Viscometer at room temperature. 10 gram cream was put into the beaker, then the viscometer rotor was put into the beaker until it was immersed in the cream. The viscometer was turned on, then read the numbers or scales listed. The tests for each formula were replicated three times.

2.2.2.4. pH Test
The cream was put into the beaker, then measured with a pH meter which had been previously calibrated with a pH 7 buffer solution. The measurements were conducted at room temperature[8].

2.2.3. Spreadability Test
A clean and dry object glass was placed on a graph paper. 0.5 g cream was placed on the glass object, then covered with another glass object, and waited for ± 5 seconds to see how much the diameter of the formed cream spread was. Furthermore, weights 50, 100, and 200 g were added on the glass object cover, and again, the diameter of the cream spread was observed. The tests for each formula were replicated three times[8].

2.2.4. Adhesive Test
0.5 g cream was placed on a clean and dry object glass, and then covered with another object glass, and given a 1 kg weight for 5 minutes. Both ends of the object glass were clamped with a clamp along
with a 50 g weight. Furthermore, the time length of the object glass to detach was observed. The tests for each formula were replicated three times[9].

2.2.5. **Cream Type Test**
0.5 g cream was placed on a clean and dry object glass, then 1 drop methylene blue solution was added, and mixed thoroughly, then covered with another object glass, and observed visually[8].

2.2.6. **Stability Test (Cycling Test)**
The cream preparation was placed inside a refrigerator at ± 4°C for 24 hours, and then removed and placed in an incubator at ± 40°C for 24 hours (1 cycle). The treatment was carried out in 6 cycles. The physical condition of the cream after the cycling test was compared to the previous condition[10].

2.3. **Cell Regeneration Ability Test via In Vivo**
The in vivo test using the experimental animals was carried out based on the permit of the Health Research Ethics Commission of the Regional General Hospital of Dr. Moewardi No. 163/I/HREC/2020. The 3-month old male Wistar white rats were divided into three groups in which each group contained three rats, namely the positive control group, negative control group, and the test group. The rats were acclimatized to the environment for one week before the test. The hair on the backs of the rats were shaved with a size of ± 4x4 cm, and cleaned with 70% alcohol. The three groups of rats were illuminated with Exoterra UV B lamp for 72 hours until erythema appeared on the rats’ skin. Over the next six days, the positive control group rats were smeared with product X (the product was claimed to be anti-aging because it was able to hide stains as well as to regenerate cells; a product by Unilever), the negative control group rats were not smeared with anything, and the test group rats were smeared with the best Potassium Azeloyl Diglycinate anti-aging cream. The observation was conducted for seven days using erythema values, namely[11]:

- 0 = No erythema
- 1 = Very few erythema with diameters ≤ 25 mm
- 2 = Clear bounded erythema with diameters of 25.10 to 30.00 mm
- 3 = Moderate to heavy erythema with diameters of 30.10 to 35.00 mm
- 4 = Erythema formed crust and were bright red with diameters ≥ 35.10 mm

2.4. **Hydration Test on Skin**
The hydration test on the skin of the respondents was carried out based on the permit of the Health Research Ethics Commission of the Regional General Hospital of Dr. Moewardi No. 163/I/HREC/2020. 12 respondents were asked to take this test. The criteria to be the respondents were: women, ages between 20 to 55 years old, no history of diseases related to allergies to the skin, and willing to be a respondent by completing the willingness form as respondents and the state control form of respondents. On the first day, the skin moisture condition of the respondents' back hand was tested using a skin analyzer tool. Furthermore, the Potassium Azeloyl Diglycinate anti-aging cream was applied on the back of the left hand with an area of ± 5x5 cm every night for one week. On the seventh day, the moisture condition of the back of the respondents' left hand was re-examined using the skin analyzer. The results of the skin moisture condition test on the back of the respondents' hand, after and before applying the Potassium Azeloyl Diglycinate anti-aging cream, were then compared[12].

2.5. **Antioxidant Activity Test**

2.5.1. **Making of 0.4-mM DPPH Main Solution**
DPPH powder, with a molecular weight of 394.32 g/mol, weighed as much as 4 mg, and put into a 25 mL volumetric flask, then methanol pro analysis was added until it reached the limit mark. The DPPH main solution was turned into homogeneous with vortex for 20 seconds, and kept at a low temperature and protected from light[13].
2.5.2. **DPPH Maximum Wavelength Determination**

2 mL of 0.4 mM DPPH main solution was added to a 10 mL volumetric flask, then methanol pro analysis was added until it reached the limit mark. The solution was vortexed for 20 seconds, incubated for 30 minutes at 37°C, then the absorption was measured at a wavelength of 400 to 600 nm using a UV-Vis spectrophotometer.

2.5.3. **Making of Blando Solution**

2 mL of 0.4 mM DPPH main solution was added to a 10 mL volumetric flask, then methanol pro analysis was added until it reached the limit mark. The solution was vortexed for 20 seconds, incubated for 30 minutes at 37°C, then the absorption was measured at the pre-obtained maximum wavelength.

2.5.4. **Pure Vitamin C Antioxidant Activity Test**

5 mg vitamin C was weighed and put in a 50 mL volumetric flask, then methanol pro analysis was added until it reached limit mark (the Vitamin C main solution was 100 ppm). The main solution was diluted into five series of concentrations, namely 1, 2, 3, 4, and 5 ppm. The concentration series was made by weighing Potassium Azeloyl Diglycinate according to the concentration, then adding 2 mL 0.4 mM DPPH and methanol pro analysis until the volume was 10 mL. All series of concentrations were vortexed for 20 seconds, and incubated for 30 minutes at 37°C, then the absorption was measured at the maximum pre-obtained wavelength.

2.5.5. **Pure Potassium Azeloyl Diglycinate Antioxidant Activity Test**

The Potassium Azeloyl Diglycinate solution was made into 5 concentrations, namely 60.000, 65.000, 70.000, 75.000, and 80.000 ppm. The concentration series was made by weighing Potassium Azeloyl Diglycinate according to the concentration, then adding 2 mL 0.4 mM DPPH and methanol pro analysis until the volume was 10 mL. The solution was vortexed for 20 seconds, and incubated for 30 minutes at 37°C, then the absorption was measured at the maximum pre-obtained wavelength.

2.5.6. **Free Radical Inhibition Percentage Calculation**

Free radical scavenger activity (antioxidant activity) of Potassium Azeloyl Diglycinate and Vitamin C were calculated as the DPPH color reduction percentage (free radical inhibition percentage) using the equation[14]:

\[
\text{DPPH Radical Inhibition (\%)} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100\%
\]

2.5.7. **IC\textsubscript{50} Value Calculation**

The IC\textsubscript{50} value was calculated from the linear regression curve between the DPPH radical inhibition percentage and the test solution concentration series (sample). The sample concentration and the DPPH radical inhibition percentage were plotted respectively on the x and y axes to obtain a linear regression equation. The equation was used to determine the IC\textsubscript{50} value. The calculation was performed using the linear regression equation; the sample concentration as the x axis, and the 50 value as the y axis[13].

3. **Result and Discussion**

3.1. **Anti-Aging Cream Physical Properties Stability Evaluation**

3.1.1. **Organoleptic Test**

Organoleptic test aims to discover the cream physical stability during storage[15]. The organoleptic test results can be seen in Table 2. Based on the table, it can be seen that the F0 cream has a rather thick consistency since there is no Potassium Azeloyl Diglycinate was added. The organoleptic test
conducted after the 12 day cycling test also gave the same results for each parameter so that all four formulations were organoleptically stable.

Table 2. Organoleptic test results of the Potassium Azeloyl Diglycinate anti-aging cream

| Storage                  | Color | Odor | Consistency | Phase Separation |
|--------------------------|-------|------|-------------|------------------|
| Cream (F0)               |       |      |             |                  |
| Before the cycling test  | White | Vanilla | Rather thick | Not              |
| After the cycling test   | White | Vanilla | Rather thick | Not              |
| Cream (F1)               |       |      |             |                  |
| Before the cycling test  | White | Vanilla | Thick       | Not              |
| After the cycling test   | White | Vanilla | Thick       | Not              |
| Cream (F2)               |       |      |             |                  |
| Before the cycling test  | White | Vanilla | Thick       | Not              |
| After the cycling test   | White | Vanilla | Thick       | Not              |
| Cream (F3)               |       |      |             |                  |
| Before the cycling test  | White | Vanilla | Thick       | Not              |
| After the cycling test   | White | Vanilla | Thick       | Not              |

3.1.2. Homogeneity Test
Homogeneity test aims to distribute the compiler components in cream[15]. Cream is said to be homogeneous if no visible coarse particles can be touched when applied to a transparent material. The homogeneity test results can be seen in Table 3.

Table 3. Homogeneity test results of the Potassium Azeloyl Diglycinate anti-aging cream

| Cream | Homogeneity           |
|-------|-----------------------|
| F0    | Homogeneous | Homogeneous |
| F1    | Homogeneous | Homogeneous |
| F2    | Homogeneous | Homogeneous |
| F3    | Homogeneous | Homogeneous |

Based on the table, it can be seen that the four cream formulations have good homogeneity, therefore, it is said to be stable in the homogeneity test.

3.1.3. Viscosity Test
Viscosity test aims to discover the cream thickness. The higher the cream viscosity, the bigger the cream resistance which makes the cream becomes more difficult to pour[16]. Based on the requirements of Indonesian National Standard (SNI) 16-4399-1996, the viscosity of good cream preparations is from 2.000 cP to 50.000 cP[17].

Table 4. Viscosity test results of the Potassium Azeloyl Diglycinate anti-aging cream

| Cream | Viscosity (cP) |
|-------|----------------|
| F0    | 18.500.00 ± 500.00 | 16.833.33 ± 763.76 |
At 25°C, Potassium Azeloyl Diglycinate has a density of 1.135 to 1.145 kg/m³; while the Aqua Dest density is 998.2 kg/m³[6]. The density of a liquid is directly proportional to its viscosity, therefore, the addition of Potassium Azeloyl Diglycinate to the cream causes an increase in the cream viscosity. This caused the F3 cream to have the greatest viscosity. After the cycling test, there was a decrease in the cream viscosity caused by glycerin and TEA which are hygroscopic, therefore, it can absorb the air moisture and increase the water volume in cream[16,18]. In addition, too high storage temperature can increase the distance between atoms, causing bonds stretching between particles, and making the cream viscosity decrease[19,20]. The statistical analysis results showed that the viscosity, before and after the cycling test for the four formulations, was significantly different (p < 0.05).

### 3.1.4. pH Test

pH test aims to determine the cream acidity degree. Based on Indonesian National Standard (SNI) 16-4399-1996, the ideal cream pH and in line with skin physiology is 4.5 to 8. Cream with too acidic pH can irritate the skin, while too alkaline pH can cause dry and scaly skin (9). The pH test results can be seen in Table 5.

| Cream | pH Before cycling test | pH After cycling test |
|-------|------------------------|-----------------------|
| F0    | 7.79                   | 7.76                  |
| F1    | 6.89                   | 6.81                  |
| F2    | 6.73                   | 6.72                  |
| F3    | 6.49                   | 6.44                  |

Potassium Azeloyl Diglycinate is a derivative of azelaic acid; the higher the Potassium Azeloyl Diglycinate addition, the lower the cream pH produced. After the cycling test, there was a decrease in pH caused by the hydrolysis reaction of the acid component which was triggered by changes in temperature during storage[21,22]. Statistical analysis showed that the pH of the four formulations, before and after the cycling test, did not differ significantly (p > 0.05).

### 3.2. Spreadability Test

The spreadability test aims to discover the cream spread ability on skin. The good spreadability cream is 5 to 7 cm[15]. Good spreadability means that the cream will be more easily applied to skin surface so that the contact between the cream and the skin becomes wider, and the active ingredients absorption into the skin is more maximum[23]. The spreadability test results can be seen in Table 6.

| Cream | Spreadability (cm) |
|-------|--------------------|
|       | Before cycling test | After cycling test |
| F0    | 6.80 ± 0.10        | 7.00 ± 0.10        |
| F1    | 6.20 ± 0.10        | 6.63 ± 0.06        |
| F2    | 6.00 ± 0.10        | 6.23 ± 0.06        |
| F3    | 5.03 ± 0.15        | 6.50 ± 0.10        |
The cream spreadability is inversely proportional to its viscosity. In the research, the F3 cream had the highest viscosity since it contained the highest concentration of Potassium Azeloyl Diglycinate, resulting in the lowest spreadability. After the cycling test, there was an increase in the dispersal power caused by a decrease in the viscosity of the four cream formulations, therefore, the fluid resistance to flow was reduced[24]. The statistical analysis results showed that the F0, F1, and F2 creams had spreadability that were not significantly different (p > 0.05), before and after the cycling test; while the F3 cream had a significantly different spreadability, before and after the cycling test (p < 0.05).

3.3. Adhesive Test

Adhesive test aims to discover how long the cream can stick to the skin. The longer the adhesive, the longer the cream will stick on the skin. The adhesive requirement for a cream preparation is not less than 4 seconds[17]. The adhesive test results can be seen in Table 7.

| Cream | Adhesive (cm) | Before cycling test | After cycling test |
|-------|---------------|---------------------|--------------------|
| F0    | 6.80 ± 0.10   | 7.00 ± 0.10         |
| F1    | 6.20 ± 0.10   | 6.63 ± 0.06         |
| F2    | 6.00 ± 0.10   | 6.23 ± 0.06         |
| F3    | 5.03 ± 0.15   | 6.50 ± 0.10         |

Adhesive is directly proportional to viscosity so that the F3 cream had the greatest adhesive. After the cycling test, there was a decrease in the adhesive of all four formulations. This decrease was directly proportional to the decrease in the viscosity of the four cream formulations caused by glycerin and TEA which are hygroscopic. The adhesive of the cream preparation containing polymers with concentrations < 3% b/b tends to decrease during storage. This is caused by the weak bonding structure in the cream system so that the cream adhesive is weakened. While the adhesive of the cream with polymer concentration > 3% b/b tends to increase, caused by the hydrogen bonds formation and the intermonomer saccharides integration that prevent polymer dissociation. In this research, the concentration of polymer (PGA) used was 0.2% b/b so that it was able to weaken the bonding between particles, and decreasing the adhesive during storage. The statistical analysis results showed that the cream adhesive, before and after the cycling test, was not significantly different (p = 0.05); while the F1, F2, and F3 cream adhesive, before and after the cycling test, were significantly different (p < 0.05).

3.4. Cream Type Test

The cream type test is done by coloring method, using a water-soluble methylene blue solution. In this research, the cream type produced was oil-in-water (O/W) so that the addition of methylene blue would produce a homogeneous color spread in the external phase of the cream. The cream type test results can be seen in Table 8.

| Cream | Cream Type | Before cycling test | After cycling test |
|-------|------------|---------------------|--------------------|
| F0    | O/W        | O/W                 |
| F1    | O/W        | O/W                 |
Based on the table, it can be seen that all four formulations have the O/W type, both before and after the cycling test. These results state that the cream is stable and does not undergo the phase inversion[25].

3.5. Cell Regeneration Ability Test via In Vivo

In vivo test aims to discover the ability of Potassium Azeloyl Diglycinate anti-aging cream in regenerating skin cells of the experimental animals. In the test, each rat’s hair was shaved with a size of ± 4x4 cm, and was exposed to Exoterra UV B lamps for 72 hours as the inflammation inducer (erythema). The positive control used was the product X whose effectiveness would be compared with the Potassium Azeloyl Diglycinate anti-aging cream. Product X contains active ingredient of Retinol-C Complex which is able to hide stains and regenerate skin cells. The Potassium Azeloyl Diglycinate anti-aging cream concentration used was 5% since it had the best physical properties stability test results. In addition, according to Sinerga (2012), the most recommended concentration of Potassium Azeloyl Diglycinate for cosmetic formulations is 5%[6]. While the negative control was given no treatment. The results of the cell regeneration ability via in vivo can be seen in Table 9 and Figure 1.

| Treatment Group | Erythema Value (Day-) |
|-----------------|-----------------------|
|                 | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| Negative control| 4  | 4  | 4  | 3  | 2  | 1  | 1  |
| Positive control| 4  | 4  | 3  | 2  | 1  | 1  | 1  |
| Potassium Azeloyl Diglycinate 5% anti-aging cream | 4  | 3  | 2  | 2  | 1  | 1  | 1  |

Figure 1. Curve of the daily erythema diameter decrease comparison on each treatment group

Based on the research, the results showed that the three treatment groups had a decrease in the erythema diameters, however, the difference was the rate of cell regeneration and the erythema diameters of each group. On the 7th day, all three treatment groups had erythema values of 1, however, there were differences in the size of the erythema diameters; the negative control group was 21.00 ± 3.61 mm; the positive control group was 17.67 ± 2.52 mm; and the test group was 4.33 ± 4.04 mm. Based on these results, it can be stated that the 5% Potassium Azeloyl Diglycinate anti-
aging cream has the best ability to accelerate the regeneration of the rats skin cells that have erythema. This is due to the ability of Potassium Azeloyl Diglycinate to relieve inflammation (erythema) in the skin cells. In addition, Potassium Azeloyl Diglycinate also has moisturizing effect so it does not irritate the skin barrier[3]. The statistical analysis results of the erythema diameters of the three treatment groups on the 7th day revealed a significance value of p < 0.05 so that there were significant differences between the treatment groups.

3.6. Hydration Test on Skin

Hydration test on the skin aims to discover the ability of the Potassium Azeloyl Diglycinate anti-aging cream in moisturizing the respondents' skin. In this test, the concentration of the Potassium Azeloyl Diglycinate anti-aging cream used was 5%. The hydration test results on the skin can be seen in Table 10.

| Respondents | Age (years) | Water content | Oil content | Water content | Oil content |
|-------------|-------------|---------------|-------------|---------------|-------------|
| 1           | 31          | 12.4          | 5.5         | 14.3          | 6.4         |
| 2           | 21          | 13.7          | 6.1         | 14.8          | 6.6         |
| 3           | 22          | 11.8          | 5.3         | 13.9          | 6.2         |
| 4           | 20          | 10.9          | 4.9         | 14.3          | 6.4         |
| 5           | 36          | 11.7          | 5.2         | 14.8          | 6.6         |
| 6           | 53          | 10.4          | 4.6         | 13.4          | 6           |
| 7           | 53          | 10.5          | 4.7         | 12.5          | 5.6         |
| 8           | 40          | 13.5          | 6           | 13.9          | 6.2         |
| 9           | 42          | 15.2          | 6.8         | 16.9          | 7.6         |
| 10          | 44          | 11.5          | 5.1         | 11.9          | 5.3         |
| 11          | 32          | 13.4          | 6           | 13.9          | 6.2         |
| 12          | 20          | 13.1          | 5.8         | 14.3          | 6.4         |

Based on the table, it can be seen that all respondents experience an increase in water content and oil content so that the 5% Potassium Azeloyl Diglycinate anti-aging cream has been shown to increase the skin's moisture. The difference in the moisture increase is influenced by the condition of each respondent, such as age, activity duration (indoors and outdoors), the amount of water consumed in a day, and the respondents' skin type (normal/sensitive). Increased moisture can also slow the wrinkles so that the skin texture becomes smoother. Statistical analysis showed a significance value of p < 0.05 for water content and oil content so that there were significant differences between the respondents' moisture, before and after using the 5% Potassium Azeloyl Diglycinate anti-aging cream.

3.7. Antioxidant Activity Test

The principle of DPPH method is the reduction of free radicals caused by the interaction between DPPH molecule and hydrogen atom released by the sample molecule compound, and is marked by a decrease in the purple color intensity in the DPPH solution[26]. The parameter used to indicate antioxidant activity is IC_{50} (inhibition concentration 50) which states the concentration of an active substance to reduce the DPPH free radical activity by 50%[27].

In this research, the antioxidant activity was tested on Potassium Azeloyl Diglycinate as the active anti-aging cream, and Vitamin C as the positive control. Vitamin C is a comparative compound that is very often used in antioxidant activity tests since it belongs to the very strong antioxidant group, and has quicker reaction to reactive oxygen compound than other substances[28].
In this research, the maximum wavelength of DPPH obtained was 516 nm with an absorbance of 0.722. The IC\textsubscript{50} value of Potassium Azeloyl Diglycinate obtained was 83.946 ppm, and Vitamin C was 1.684 ppm. These results indicate that Potassium Azeloyl Diglycinate is a very weak antioxidant group (IC\textsubscript{50} > 200 ppm), while Vitamin C is a very strong antioxidant group (IC\textsubscript{50} < 50 ppm). The cause of the low antioxidant activity of Potassium Azeloyl Diglycinate is because the structure only has 1 hydrogen atom that can be donated, while Vitamin C has 4 hydrogen atoms that can be donated.

4. Conclusion
4.1. The formulation of 5% Potassium Azeloyl Diglycinate anti-aging cream has the best stability and physical properties evaluation according to the standard, after a 12 day cycling test.
4.2. The 5% Potassium Azeloyl Diglycinate anti-aging cream can provide better skin cells regeneration effect on the rats with erythema than the positive and negative control groups. This is stated by the erythema diameter of the test group, which is 4.33 ± 4.04 mm, while the positive control is 17.67 ± 2.52 mm, and the negative control is 21.00 ± 3.61 mm. The 5% Potassium Azeloyl Diglycinate anti-aging cream can also increase the respondents' skin moisture. This is stated through the increase in the water and oil content of 12 respondents who participated in this research.

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