Antibacterial cream formulation of ethanolic Pliek U extracts and ethanolic residue hexane Pliek U extracts against Staphylococcus aureus

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Abstract. Indonesia as the largest archipelago country in the world is also known as a country with plenty of natural resources that can be used as traditional medicinal plants. Pliek U is a fermented crude coconut meat has been used as an ingredient in Aceh traditional food and also used as a traditional topical medicine in Aceh. Pliek U is potential as a source of free fatty acids (FFAs) such as lauric acid and monolauric acid which have antimicrobial properties. The aim of this study is to determine the activity ethanolic Pliek U extracts (EPUE) and ethanolic of residue hexane of Pliek U extracts (ERHPUE) and also the activities of their cream formulation against Staphylococcus aureus. The antibacterial activity was determined by disc diffusion method with clindamycin as a positive control. The antibacterial activity results showed that EPUE has antibacterial activity against Staphylococcus aureus with diameter inhibition zone of 8.66±0.57 mm, while ERHPUE and their cream have no activity against Staphylococcus aureus.

1. Introduction

The Acehnese people have traditionally processed the coconut into traditional coconut oil for consumption. The fermented coconut meat is then dried and processed into coconut products such as Pliek U [1]. Coconut is rich in fatty acids such as lauric acid which has an antibacterial effect against Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis [2].

The research that has been done by Nurliana et al. in 2009 concluded that ethanolic Pliek U extract and ethanolic of residue hexane of Pliek U extracts able to inhibit the growth of Staphylococcus aureus with MIC₅₀ values of 19.33 ± 0.47 mm and 18.33 ± 0.47 mm respectively [3].

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*Staphylococcus aureus* is known to be a major bacterial in the complication of atopic dermatitis. Up to now, for the management of atopic dermatitis, there is no scientific evidence that shows *Pliek U* extract is effective moisturizer for adult patients with atopic dermatitis.

The Development of *Pliek U* as a therapeutic substance candidate should be continued by formulating this substance in pharmaceutical form. One of the appropriate forms is cream, because it can be smeared easily on the skin and can be absorbed easily. Based on the description above, the aim of this study is to analyze the activity ethanolic *Pliek U* extract (EPUE) and ethanolic of residue hexane of *Pliek U* extracts (ERHPUE) and also the activities of their cream formulation against *Staphylococcus aureus*.

2. Methods

2.1 Samples collection

Samples were obtained from the household production located in Gampong Paya Bieng, Bireuen, Aceh Province, Indonesia. The sample obtained is *Pliek U*.

2.2 Processing of ethanolic *Pliek U* extracts (EPUE)

The *Pliek U* extraction was performed by macerating 250 g of *Pliek U* with 1500 mL ethanol 96% and being shaken at a speed of 130 rpm at 28 °C for 48 hours. It is then filtered using a fritted glass filter connected to a vacuum pump. The *Pliek U* residue was re-extracted in a separating funnel twice using 300 mL of ethanol 96%. The obtained filtrate was concentrated by using a rotary evaporator at 50 °C to obtain ethanolic *Pliek U* extracts (EPUE).

2.3 Processing of ethanolic of residue hexane of *Pliek U* extracts (ERHPUE)

Extraction of *Pliek U* was done by macerating 250 g of *Pliek U* with 1500 mL hexane solvent with a ratio of 1:6, then shaked with a speed of 130 rpm at 28°C for 48 hours. It is then filtered using a fritted glass filter connected to a vacuum pump. Filtering results obtained by macerate and residue. The residue was re-extracted using 300 mL of hexane solvent in the separation funnel twice to separat hexane layer and the residue. The residue is then re-extracted using 1500 mL ethanol 96% in the same procedure. The ethanol macerate was concentrated using a rotary evaporator at a temperature of 50 °C, to obtain the ethanolic of residue hexane of *Pliek U* extracts (ERHPUE).

2.4 Formulation and processing of cream

Formulations of cream are made using vanishing cream as a base with an ingredients namely stearic acid, cera alba, vaseline album, triethanolamine, propylenglycol, and aquadest. The cream is made of 2 formulations and each formulation is made up to 3 preparations with a weight in each preparation is 100 g. The cream formulation is made ie F₀ which does not contain extract and F₁ contains 4%.

| Materials                  | F₀ Concentration (%) | F₁ Concentration (%) |
|----------------------------|----------------------|----------------------|
| EPUE/ERHPUE                | -                    | 4                    |
| Stearic acid               | 14.2                 | 14.2                 |
| Cera alba                  | 2                    | 2                    |
| Vaseline album             | 8                    | 8                    |
| Triethanolamine            | 1.5                  | 1.5                  |
| Propylenglycol             | 8                    | 8                    |
| Aquadest                   | ad 100               | ad 100               |

2.5 Evaluation of EPUE/ERHPUE cream

Evaluation of cream includes cycling test, organoleptic, homogeneity, pH, dispersion, adhesion and viscosity.
2.5.1 Cycling test. The cream is put into a plastic pot and covered with aluminum foil. The cream is placed in the oven at 45 ± 2°C for 24 hours, then placed in the freezer at 5 ± 2°C for 24 hours (1 cycle). This cycle is done as much as 6 cycles for 12 days [4].

2.5.2 Organoleptic test. Observations made visually on the cream include the shape, color, odor, and the presence or absence of separation between the cream base and EPUE/ERHPUE [5].

2.5.3 Homogeneity test. 0.5 g cream was applied to object glass and then the particle size was observed [6].

2.5.4 pH test. The cream is weighed as much as 1 g and put into the beaker, then added 10 mL of distilled water and stirred. pH meters that have been calibrated with aquades (pH 7.0) are fed into the solution and pH was read on the monitor.

2.5.5 Dispersion. A total of 1 g of cream is placed between two round glasses. A load of 5 g is placed on the glass and left for 1 minute. The diameter of the cream distribution is measured. The other load continuously added after 1 minute with a weight of 5 g to obtain a constant diameter of cream distribution. The recommendation of dispersion is 5 - 7 cm [5].

2.5.6 Viscosity. 100 g cream is put in a beaker and placed under the International Rheology Viscometer. Then the spindle is inserted into the cream and the viscosity of the cream is measured. The recommendation of viscosity is 5000 - 20000 mPa [7].

2.6 Culture of Staphylococcus aureus. The identification process of Staphylococcus aureus, the manufacture of Staphylococcus aureus media and regeneration was performed at the Microbiology Laboratory, Faculty of Medicine, Syiah Kuala University, Darussalam, Banda Aceh, Indonesia.

2.7 Antibacterial activity test. The antibacterial activity test of the cream was done by disk diffusion method. The advantages of the disk diffusion method are easy performance and less requirement of special equipment [8].

2.8 Processing of EPUE 4% and ERHPUE 4% and their cream formulation. EPUE 4% and ERHPUE 4% were prepared by dissolving 0.4 g of EPUE and 0.4 g ERHPUE in 10 ml ethanol, then soaked the disc for 30 minutes. Each 1 g of EPUE 4% cream and ERHPUE 4% was soaked in 1 mL sterile distilled water and then soaked in a blank disc for 30 minutes.

2.9 Antibacterial activity test of EPUE 4%, ERHPUE 4% and their cream formulation. The antibacterial activity test was performed with seven types of test samples: EPUE 4%, ERHPUE 4%, F0 (cream of EPUE 4% and cream of ERHPUE 4%), clindamycin (positive control), ethanol 96% (negative control) and cream F0 / base (negative control). The antibacterial activity test method was performed using the disc method. The discs are then immersed in six sample types: EPUE 4%, ERHPUE 4%, F0, F1 and ethanol 96% above the surface of MHA media. The bacterial inoculum is scratched on MHA media using a sterile cotton bud. Next, put the disc paper soaked in EEPU 4%, EERHPU 4%, F0, F1 and ethanol 96% above the surface of MHA media. The positive control used is clindamycin disc.

3. Result and Discussion

3.1 Sample extraction
The organoleptic characteristics of ethanolic Pliek U extracts (EPUE) obtained are blackish brown color, typical smell of Pliek U and its consistency is thick. The amount of EPUE obtained was 60 g with a rendemen percentage of 24%. The EPUE obtained then was tested for its activity against Staphylococcus aureus, and also was used to prepare its cream.

The ethanolic of residue hexane of Pliek U extracts (ERHPUE) obtained was 52.2 g with an extraction rate up to 20%. Its organoleptic features are dark brown, typical smell of Pliek U and thick.
3.2 Evaluation of cream

Based on Table 2 it can be seen that in F0 and F1 creams there is no change either the color, odor, shape, or separation. The F0 and F1 creams remain homogeneous from prior to the cycling test up until the 6th cycle. The pH value of the F0 and F1 is above 6.5, with the highest pH reached 8.1 (cream base) when cycling test 0 and 1, the lowest pH 6.7 (ERHPUE cream) was reached at the 6th cycle. Spreading well at no load, 50 g and the highest 100 grams when cycling test 0 with a value of 6.2; 6.6 and 7.0 (cream base) respectively, and lowest dissipation is 3.1; 3.2 and 3.5 (ERHPUE cream) when 5th and 6th cycle No change shows that the creams of F0 and F1 are stable during storage at two different temperatures and the cream is homogeneous if the distribution of particles is evenly distributed and there is no breakdown in the cream.

| Cycle | Organoleptic | pH | Dispersion (cm) |
|-------|--------------|----|----------------|
|       | Color | Odor | Shape | Separation | Homogeneity |     |     |     |
| 0     | EPUE  | F0   | -     | -     | -     | Homogeneous | 8.1 | 6.2 | 6.6 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.1 | 4.1 | 4.5 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 8.1 | 5.5 | 6.6 |
|       |       | F1   | -     | -     | -     | Homogeneous | 6.8 | 4.1 | 4.2 |
| 1     | EPUE  | F0   | -     | -     | -     | Homogeneous | 8.1 | 6.0 | 6.5 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.0 | 4.0 | 4.4 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 8.1 | 5.4 | 5.8 |
|       |       | F1   | -     | -     | -     | Homogeneous | 6.9 | 4.0 | 4.1 |
| 2     | EPUE  | F0   | -     | -     | -     | Homogeneous | 8.0 | 5.9 | 6.4 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.0 | 3.9 | 4.2 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 8.0 | 5.2 | 5.7 |
|       |       | F1   | -     | -     | -     | Homogeneous | 6.9 | 3.8 | 4.0 |
| 3     | EPUE  | F0   | -     | -     | -     | Homogeneous | 8.0 | 5.8 | 6.2 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.0 | 3.9 | 4.2 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 8.0 | 5.0 | 5.4 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.1 | 3.6 | 4.0 |
| 4     | EPUE  | F0   | -     | -     | -     | Homogeneous | 8.0 | 5.7 | 6.1 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.0 | 3.8 | 4.1 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 8.0 | 5.0 | 5.4 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.0 | 3.4 | 3.6 |
| 5     | EPUE  | F0   | -     | -     | -     | Homogeneous | 7.9 | 5.7 | 6.0 |
| 6     | EPUE  | F0   | -     | -     | -     | Homogeneous | 7.9 | 5.6 | 6.0 |
|       |       | F1   | -     | -     | -     | Homogeneous | 7.9 | 5.0 | 5.1 |
|       | ERHPUE| F0   | -     | -     | -     | Homogeneous | 7.9 | 5.0 | 5.1 |
|       |       | F1   | -     | -     | -     | Homogeneous | 6.7 | 3.1 | 3.2 |

- : no change.
F0 : cream base.
F1 : cream formulation.

The acidity of the cream affects the acceptance of the cream onto the skin. The pH of the cream should be in accordance with the pH of the skin which is between 4.5-6.5. Too-alkaline pH preparations cause scaly skin, whereas too acid pH will cause skin irritation [9]. The pH of F0 and F1 does not satisfy the expected pH value due to the pH value of the cream F0 and F1 exceeds the maximal threshold. The pH value of F1 is lower than the pH value of F0. The difference in pH values between F0 and F1 can be due to the pH of EPUE is 4.0 (acid) and ERHPUE is 4.1 (acid). The pH of the F0 and F1 cream decreased during storage caused by hydrolysis of the acidic compound due to the temperature change during the cycling test [10]. Spreading test is done to know the cream ability to be evenly spread on skin. Recommended spread of cream is 5 - 7 cm. Cream that has a spreading power with a range of 5 - 7 cm will be easily applied and spread on the skin [5].
F₁ distribution does not meet the requirement where the spreading power of F₁ is 3.1 - 4.9 cm (EPUE) and 3.1 - 4.3 (ERHPUE), meanwhile the spreading energy of F₀ is qualified with the diameter of cream distribution in the range of 5.6 - 7.0 cm. Factors that affecting the power of spread is the addition of extracts. The addition of extract to the cream causes the volume of water used to be less so that the consistency of the cream becomes tough, resulting in the spread of cream oil becomes decreased [10]. Therefore, the dispersion energy of the F₁ cream (containing EPUE and ERHPUE) is smaller than the spreading power of F₀. The distribution of F₀ and F₁ creams is smaller after cycling test which can be affected by viscosity, where the viscosity of F₀ and F₁ is greater after cycling test.

The viscosity value is related with whether or not the sample is easy to be removed from the container. When the sample is easily removed from the containe, the viscosity value meets the packaging requirements, and it can be easily used [5]. The viscosity test results can be seen in Table 3. Recommended viscosity values is between 5000-20000 mPa [7]. The viscosity values of F₀ and F₁ are qualified to be considered as a good viscosity for cream.

| Table 3. Viscosity test |
|-------------------------|
| Cycle | Cream | Viscosity (mPa) |
| EPUE | Before cycling | F₀ | 5182.44 |
| | After cycling | F₀ | 5803.64 |
| | Before cycling | F₁ | 7366.22 |
| | After cycling | F₁ | 7982.17 |
| ERHPUE | Before cycling | F₀ | 7562.94 |
| | After cycling | F₀ | 9134.37 |
| | Before cycling | F₁ | 7730.04 |
| | After cycling | F₁ | 9458.49 |

F₀ : cream base.
F₁ : cream formulation.
Recommanded viscosity value : 500-20000 mPa.

3.3 Antibacterial activity test
Inhibitory zone of EPUE 4%, ERHPUE 4%, F₁(cream of EPUE 4% and ERHPUE 4%), ethanol and F₀ (negative control), and clindamycin (positive control) is exhibited in table 4.

| Table 4. Antibacterial test |
|-----------------------------|
| Samples | Diameters of the inhibitory zone (mm) | D_{mean} | Classification (Morales et al) |
| | D₁ | D₂ | D₃ |  |
| F₁ (cream of EPUE 4%) | 9.0 | 8.0 | 9.0 | 8.66±0.57 | Moderate |
| EEPU 4% | - | - | - | 0 |
| F₁ (cream of ERHPUE 4%) | - | - | - | 0 |
| ERHPUE 4% | 15 | 14.0 | 14.66±0.57 | Strong |
| Control + (clindamycin) | 15 | 14.0 | 14.66±0.57 | Strong |
| Control – (ethanol 96%) | - | - | - | 0 |
| Control – (F₀/base cream) | - | - | - | 0 |

The mean inhibitory zone diameter of EPUE 4% is 8.66 ± 0.57 mm which, according to Morales et al.,2003, the inhibitory zone includes moderate antibacterial activity [11]. The absence of 4% ERHPUE activity against Staphylococcus aureus indicates that suspected fatty acid compounds that have anti-bacteria activity in Plik U have been extracted into hexane solvent. Fatty acid compounds such as lauric acid, capric acid, myristic acid, palmitic acid, oleic acid and linoleic acid are nonpolar and the hexane solvent is also nonpolar which attracts nonpolar compounds, so there is no inhibitory zone in ERHPUE 4%. In contrary with Nurliana et al. (2009)
which suggested that the inhibitory zone diameters of EPUE and ERHPUE (crude extract) against *Staphylococcus aureus* are 19.33 ± 0.47 and 18.33 ± 0.47 mm respectively and were classified as a strong antibacterial activity [3] . The difference between this study and Nurliana's research were due to different concentration of the samples tested. EPUE 4% cream and ERHPUE 4% cream also do not have an inhibitory zone formed because the cream can not diffuse well into the disc so that no inhibition zone is formed. Positive control (clindamycin) resulted in an inhibitory zone of 14.66 ± 0.57 mm which was a strong activity. The negative control used was 96% ethanol as a 4% EEPU counter and base cream (F0) as a comparison of 4% EPUE cream and 4% ERHPUE (F1). Negative control (ethanol and base cream) did not show any antibacterial activity characterized by there is no inhibition zone. These results suggest that ethanol solvent and cream base (F0) does not affect the antibacterial test results of the extract and their cream.

4. Conclusion
Based on the results of the study it can be concluded that EPUE 4% has antibacterial activity against *Staphylococcus aureus*, while ERHPUE 4% and their cream do not have antibacterial activity against *Staphylococcus aureus*.

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