Formulation of Anti-acne Cream Bawang Dayak Ethanol Extract (*Eleutherine bulbosa* (Mill.) Urb.)

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Abstract. Bawang dayak (*Eleutherine bulbosa* (Mill.) Urb.) is a typical plant of Central Kalimantan. In the previous studies, it was known that bawang dayak ethanol extract can inhibit *Propionibacterium acnes,* and was made in the cream formulation to improve the efficiency of using traditional medicine. This study was aimed to determine the effect of bawang dayak cream storage for 30 days on physical properties and antibacterial activity compared to the preliminary studies conducted on the cream on days 0 and 7. Four formulas of cream were made by a variation of bawang dayak ethanol extract. F1 5%, F2 10%, F3 15%, and F4 20%. Then evaluated by organoleptic, homogeneity, pH, adhesion, and dispersion test. Cream potential as anti-acne was determined by antibacterial activity test against *Propionibacterium acnes*, using a disc-diffusion technique. The results showed that F3 dan F4 cream meets the requirements for pH, adhesion, and dispersion test, but not homogeneous. The adhesion time in this study was better than the formula on day 7. All cream formula on the 30 days of storage showed weak activity against *Propionibacterium acnes.* This present study showed the potential of all formula as anti-acne cream but further research needed to improved formula composition and stability so it can be developed as an anti-acne cream product.

Keyword: anti-acne cream, bawang dayak, *Propionibacterium acnes,* physical properties

1. Introduction

Acne (*acne vulgaris*) is a skin disease that attacks the pilosebaceous skin, namely the sebaceous glands and hair follicles. Acne causes noninflammatory lesions (open and closed comedones), inflammatory lesions (papules, pustules, and nodules), and varying degrees of scarring. Acne formation occurs due to follicle blockage by dead cells, sebum and inflammation caused by *Propionibacterium acnes* in sebaceous follicles [1,2]. *P. acnes* was involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis [3].

Bawang dayak (*Eleutherine bulbosa* (Mill.) Urb.) is a typical plant of Central Kalimantan. This plant has been used for generations by the Dayak people as traditional medicine. Empirically, the bulb part of bawang dayak is known to have properties to treat ulcers or skin diseases. Active compounds contained in bawang dayak bulb that can provide antibacterial activity include alkaloids, glycosides, flavonoids, phenols, steroids, and tannins [4,5].

In the previous studies, it was known that bawang dayak ethanol extract can inhibit *Propionibacterium acnes,* and was made in the cream formulation to improve the efficiency of using

traditional medicine. The results of the homogeneity test on day 7 were separate and non-homogenous (F3 and F4), but all formula pH suitable for topical application. On day 0, F1 and F2 shown antibacterial activity in the category weak activity; F3 and F4 in the category moderate activity, while on day 7 all formula have antibacterial activity in the category weak activity [6]. This study was aimed to determine the effect of bawang dayak cream storage for 30 days on physical properties and antibacterial activity compared to the preliminary studies conducted on the cream on days 0 and 7.

2. Material and Methods

2.1. Collection of plant

Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) were collected from Sei Gohong Village, Bukit Batu Sub-District, Palangka Raya, Central Kalimantan, Indonesia.

2.2. Preparation of plant extract

The plant materials were dried under the sun for 5-7 days. The dried plant materials were crushed by grinder. The powder of the plant materials was extracted with 96% ethanol using percolator, and once process was finished, all extracts were concentrated in ad rotary evaporator.

2.3. Formulation preparation

The formulation components used are listed in Table 1. The components consist of oil-soluble (stearic acid, adeps lanae, and paraffin liquid) and water-soluble (triethanolamine, nipagin, and aquadest). Each solution – oil soluble and water soluble – heated up to 55°C until melts. Ethanolic extract of bawang dayak dissolved in aquadest, then put into water-soluble and stir until homogeneous in a mortar, oil-soluble add gradually, stir until the cream base was formed. The last add oleum roses and stir ad homogeneous [6].

Table 1. Formulation of an anti-acne cream bawang dayak ethanol extract.

Material	Amount (mg)			
	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)	Formula 4 (F4)
Bawang dayak ethanol extract	1250 (5%)	2500 (10%)	3750 (15%)	5000 (20%)
Ol. Rosae	15 gtt	15 gtt	15 gtt	15 gtt
Oily-phases				
Stearic acid	5000	5000	5000	5000
Adeps lanae	750	750	750	750
Paraffin liquid	6250	6250	6250	6250
Aqueous-phases				
Triethanolamine	375	375	375	375
Nipagin	25	25	25	25
Aquadest ad	25000	25000	25000	25000

2.4. Physical property tests on cream

2.4.1. Organoleptic test. Cream preparations that have been made are observed in color, odor, and texture. The experiment was replicated 3 times.

2.4.2. Homogeneity test. The particle size was observed on the slide to find the coarse particles. Preparations should show a homogeneous composition and no visible coarse grains [7].

2.4.3. pH test. Determination pH of the preparation is done using a pH meter [8].

2.4.4. Adhesion test. A total of 0.5 g of preparation were spread on the disc glass, on top of it, other glass objects placed and pinned under 1 kg load for 1 min. Then, disc glass mounted on test equipment, the load is released, and the time was recorded up to the second object of the glasses falling off [9].

2.4.5. Dispersion test. Cream with 0.5 g was placed in the middle of a round glass scale. Round glass which has been weighted placed thereon and left for 5 min. After that followed with 50 g load, let stand for 1 min and record the diameter of the spread cream, did the same thing with 100 g and 150 g [9].

2.5. Antibacterial activity test against Propionibacterium acnes

A cream formula was tested to determine an antibacterial activity against *Propionibacterium acnes,* using a disc-diffusion technique with four variations of concentration of 5% (F1), 10% (F2), 15% (F3), and 20% (F4). The McFarland 0.5 standard prepared and 10 mL put into sterile tubes. The bacterial suspension made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1-2x10^8 CFU/mL (according to McFarland 0.5 standard). A sterile cotton swab was immersed in a standardized bacterial suspension and was used to evenly inoculate on Mueller-Hinton agar plate. Then, all the discs that immersed in the cream formula of bawang dayak ethanol extract placed on the plates. A clindamycin antibiotic used as positive controls with concentration variations of 0.5%, 1%, 2%, and 4%. Discs that immersed in clindamycin also placed on the plates. The plates then incubated for 24 h at 37°C. The diameter of the zone of inhibition formed was measured in mm using a caliper. The study repeated in triplicates for each cream formula and positive control.

3. Results and Discussion

3.1. Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) *bulb extract.*

From 5.3 kg of bawang dayak bulb percolated with 96% ethanol solvent produced 315.6 grams of thick extract, extract yield of 5.95% w/w.

3.2. Physical property tests on cream

3.2.1. Organoleptic test. The result of the organoleptic test showed that F1 had a lighter brown color and odor than other formulas (Table 2). The difference in color is due to differences in the concentration of bawang dayak ethanol extract in the four formulas.

Table 2. The result of organoleptic test.

Formula (F)	Color	Odors	Texture
1	Light brown	Thypical of bawang dayak	Semi-solid
2	Brown	Thypical of bawang dayak	Semi-solid
3	Dark brown	Thypical of bawang dayak	Semi-solid
4	Dark brown	Thypical of bawang dayak	Semi-solid

3.2.2. Homogeneity test. This study showed that on day 30, F1 and F2 were homogeneous, but F3 and F4 showed a separation phase between the oil phase and the aqueous phase (Table 3). In a previous study, it was known that on day 0 all formulas were homogeneous, while on day 7 F3 and F4 were not homogeneous, which was indicated by the separation of the oil phase dan the aqueous phase [6]. The homogeneity test of the cream aims to see whether all the content are combined perfectly. This homogeneity is related to the suitability of the dosage at each application. If the ingredients are not perfectly combined there may be differences in dosage or content in each formulation that is applied.

Table 3. The result of homogeneity test.

Formula (F)	Characteristic	Homogeneity
1	No coarse grain	Homogeneous
2	No coarse grain	Homogeneous
3	There are coarse grain	Not homogeneous
4	There are coarse grain	Not homogeneous

3.2.3. pH test. Table 4 showed the result of a pH test on day 30, where F1 was 5.6 and the other formula was 6. The pH obtained in this study is nearly the same as the results of previous studies conducted on days 0 and 7 [7]. The pH that suitable for topical application is the same with skin pH, between 4.5-6 [10].

Table 4. The result of pH test.

Formula (F)	pH (mean ± SD; n=3)
1	5.6 ± 0
2	6 ± 0
3	6 ± 0
4	6 ± 0

3.2.4. Adhesion test. The results showed that the ability of the cream to adhere to the skin was more than 4 s for F3 and F4 (day 30) (Table 5). In the previous study was known that the adhesion test of cream on day 0 was more than 4 s, while the time of adhesion decrease on day 7 [7]. The adhesion test of the cream serves to find out how long the cream adheres to the skin surface. The longer the cream attached to the skin, the more active substance is absorbed. A cream meets the requirements if it has adhesion for more than 4 s [11].

Table 5. The result of adhesion test.

Formula (F)	Adhesion time (s) (mean ± SD; n=3)
1	1.9 ± 1.0
2	2.6 ± 2.0
3	4.6 ± 2.5
4	4.1 ± 1.2

3.2.5. Dispersion test. The dispersion test aims to determine the softness of the cream mass so that it can be seen the ease of applying the preparation to the skin. The requirements for the dispersion test with the addition of a final load of between 5-7 cm [12]. In this study, it is known that F2 does not meet the requirements because it has dispersive power of less than 5 cm (Table 6). In a previous study, on days 0 and 7, F1 and F3 met the requirements for the dispersion test [7].

Table 6. The result of dispersion test.

Formula (F)	Dispersive power (cm) (mean ± SD; n=3)
1	6.4 ± 0.2
2	4.6 ± 1.2
3	5.6 ± 0.7
4	5.3 ± 0.8

3.3. Antibacterial activity test against Propionibacterium acnes

The antibacterial activity can be classified into three levels: weak activity (inhibition zone lower than 12 mm), moderate activity (inhibition zone between 12 and 20 mm), and strong activity (inhibition zone higher than 20 mm) [13]. In this study, the antibacterial activity test of the cream against *Propionibacterium acnes* was done in triplicates. Accordingly, all cream formula of bawang dayak ethanol extract on day 30 have weak activity against *Propionibacterium acnes*, whose inhibition zones were in the range of 5.6 ± 1.4 – 9.6 ± 2.1 mm (Table 7 and Figure 1). In the previous study, the inhibitory test results on day 0 F1 have 7.83 mm inhibition zone (weak activity), F2=9.53 mm (weak activity), F3=12.47 mm (moderate activity), and F4=12.53 mm (moderate activity), while all formula which stands until 7 days have decrease zone of inhibition in category weak activity. Inhibition zone decrease on day 7 may be caused storage not in a cool area like in a refrigerator, so it was needed to develop the research further [7].

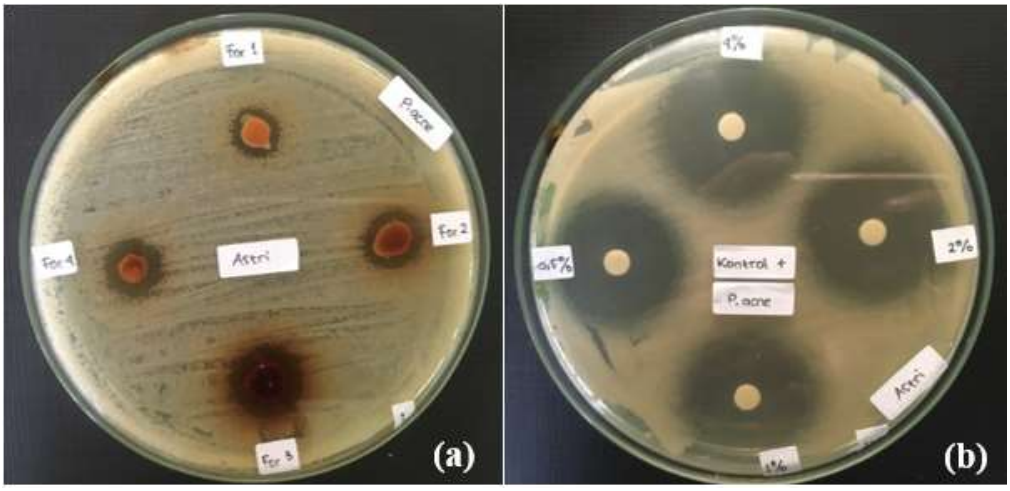

Figure 1. Antibacterial activity of cream formula of bawang dayak ethanol extract (a) and clindamycin as a positive control (b).

Table 7. Zone of inhibition of bawang dayak cream.

Formula (F)	Zone of inhibition (mm) (mean ± SD; n=3)
1	5.6 ± 1.4
2	7.8 ± 1.2
3	9.6 ± 2.1
4	8.9 ± 1.5

Table 8. Zone of inhibition of clindamycin.

Concentration (%)	Zone of inhibition (mm) (mean ± SD; n=3)
0.5	24.6 ± 0.7
1	26.9 ± 3.2
2	27 ± 0.6
4	28.8 ± 0.9

In this study, clindamycin was used as a positive control. Clindamycin was known as one of the antibiotics used for acne treatment [14]. The inhibition zones produced by clindamycin with

concentration 0.5%, 1%, 2%, and 4% against *P. acnes* were 24.6±0.7 mm, 26.9±3.2 mm, 27±0.6 mm, and 28.8±0.9 mm, respectively as presented in Table 8 and Figure 1. This research showed that all cream formula with different concentration of bawang dayak ethanol extract has the potential to inhibit *P. acnes* that caused acne vulgaris although zone of inhibition smaller than clindamycin. The secondary metabolites such as flavonoids, alkaloids, saponins, and tannins contained in bawang dayak ethanol extracts can be responsible for the antibacterial activity against *P. acnes* [7].

4. Conclusion

The results showed that F3 dan F4 cream meets the requirements for pH, adhesion, and dispersion test, but not homogeneous. The adhesion time in this study was better than the formula on day 7. All cream formula on the 30 days of storage showed weak activity against *Propionibacterium acnes.* This present study showed the potential of all formula as anti-acne cream but further research needed to improved formula composition and stability so it can be developed as an anti-acne cream product.

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