

ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT BAWANG DAYAK (*ELEUTHERINE BULBOSA* (MILL.) URB) IN CREAM AGAINST *PROPIONIBACTERIUM ACNES*

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ABSTRACT

Objective: The aims of this present study were to formulate antiacne cream consisting ethanolic extract of bawang dayak and evaluate antibacterial activity of cream on day 0 and day 7 to see stability activity and preparation.

Methods: Cream formula of bawang dayak was evaluated organoleptic characteristics, homogeneity, pH, adhesion test, dispersion test, and *in vitro* antibacterial against *Propionibacterium acnes*.

Results: The results of evaluate cream homogeneity were F3 and F4 on day 7 separate and non-homogen but all formula pH suitable for topical application. On day 0, F1 and F2 shown antibacterial activity in category weak activity, F3 and F4 in category moderate activity, while on day 7 all formula have antibacterial activity in category weak activity.

Conclusion: All cream formula potentials inhibit against *P. acnes* but this research must be improved both of preparation and stability activity.

Keywords: Acne vulgaris, Bawang dayak, Cream, *Eleutherine bulbosa*, *Propionibacterium acnes*.

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INTRODUCTION

Acne vulgaris is one of the problems experienced by teenagers and adults because in this modern era, physical appearance is one of the most important things that must be considered. Acne is not a serious disease, but it can lead depression and loss of confidence. There are many causes of acne such as hormone level, fat or oil in skin, and a bacterium like *Propionibacterium acnes* [1].

Many cosmetic products that offer to improve acne problems, nature also provides a solution to these [2]. Bawang dayak is one of traditional medicines in Central Kalimantan. Based on research before, extract ethanol of bawang dayak can inhibit *P. acnes*, so in this research, ethanol extract was made in cream formulation to improve the efficiency of using traditional medicine.

METHODS

Collection of plant

Fresh plant materials, bulbs (20 kg) of bawang dayak (*Eleutherine bulbosa* (Mill.) Urb) were procured from farmer cultivation in Sei Gohong, Bukit Batu Palangka Raya Central Kalimantan.

Preparation of plant extracts

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 perkolator, and once process was finished, all extracts were concentrated in a rotary evaporator.

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 (TEA, 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 homogenous in mortar, oil soluble add gradually, stir until the cream base was formed. The last add oleum roses and stir ad homogenous.

Evaluation of cream

Organoleptic properties

The cream was observed for color, odor, and appearance.

Homogeneity observed

The particles size was observed on the slide to find the coarse particles. Preparations should show a homogeneous composition and no visible coarse grains [3].

PH measurements

Determining pH of the preparation is done using pH meter [4].

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 [5].

Adhesion test

A total Of 0.5 g of preparation were spread on the disc glass, on top of it, other glass object placed and pinned under 1 kg load for 1 min. Then, disc glass mounted on test equipment, load is released, and the time was recorded up to the second object of the glasses falling off [5].

Evaluation of antibacterial activity by zone of inhibition by well diffusion method

Ethanolic extract of bawang dayak was evaluated for *in vitro* antibacterial activity against *propionibacterium acnes* using the disc diffusion method with different concentrations (F1=5%, F2=10%, F3=15% and F4=20%) of extract bawang dayak in a cream formula.

Table 1: Various cream formula of ethanolic extract bawang dayak

Materials	F1	F2	F3	F4
Extract ethanolic of bawang dayak	5% (1250 mg)	10% (2500 mg)	15% (3750 mg)	20% (5000 mg)
Ol. Rosae	12 gtt	12 gtt	12 gtt	12 gtt
Oily phases (mg)				
Stearic acid	5000	5000	5000	5000
Adeps lanae	750	750	750	750
Paraffin liquid	6250	6250	6250	6250
Aqueous phase (mg)				
Triethanolamine	375	375	375	375
Nipagin	25	25	25	25
Aquadest ad	25,000	25,000	25,000	25,000

Table 2: Organoleptic appearance of various cream formula bawang dayak

Observation	Color	Odor	Appearance
Day 0			
F1	Brown (+)	Significant (+++)	Semi-solid
F2	Brown (++)	Significant (+++)	Semi-solid
F3	Brown (+++)	Significant (++++)	Semi-solid
F4	Brown (+++)	Significant (++++)	Semi-solid
Day 7			
F1	Brown (++)	Significant (+++)	Semi-solid
F2	Brown (+++)	Significant (+++)	Semi-solid
F3	Brown (++++)	Significant (++++)	Semi-solid
F4	Brown (++++)	Significant (++++)	Semi-solid

+: Weak, ++: Moderate, +++: Strong, ++++: Very strong

Table 3: Homogeneity test of various cream formula bawang dayak

Observation	Result
Day 0	
F1	Homogen
F2	Homogen
F3	Homogen
F4	Homogen
Day 7	
F1	Homogen
F2	Homogen
F3	Non-homogen
F4	Non-homogen

Table 4: Adhesion test of various cream formula bawang dayak

Observation	Adhesion (seconds)
Day 0	
F1	4.24
F2	4.67
F3	4.85
F4	4.55
Day 7	
F1	2.17
F2	2.76
F3	2.93
F4	2.43

The bacterial isolates were subcultured into a nutrient. The 24-hour-old bacterial culture was standardized using McFarland standard (10^6 CFU/mL of 0.5 McFarland standard). Mueller-Hinton agar (MHA) was used for bacteria bioassay. MHA was prepared by dissolving 38 g in 1000 ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121°C for 15 min [6].

MHA plates were prepared and bacterial strains were inoculated by cotton swab, and then, antibiotic and cream with various concentrations of extract bawang dayak applied in it. The plates were incubated at 37°C

Table 5: Dispersion test of various cream formula bawang dayak

Observation	First (cm)	50 g (cm)	100 g (cm)	150 g (cm)
Day 0				
F1	3.56	5.22	5.72	6.20
F2	3.03	3.49	3.78	4.07
F3	3.80	4.53	5.06	5.40
F4	3.36	4.05	4.49	4.93
Day 7				
F1	4.04	4.52	4.98	5.41
F2	3.43	4.20	4.43	4.93
F3	3.86	4.48	4.97	5.15
F4	3.27	3.51	4.04	4.38

Table 6: PH test of various cream formula bawang dayak

Observation	pH	Result (pH=4.5-6)
Day 0		
F1	6	Qualified
F2	6	Qualified
F3	6	Qualified
F4	6	Qualified
Day 7		
F1	5.7	Qualified
F2	6	Qualified
F3	6	Qualified
F4	6	Qualified

Table 7: Zone of inhibition of clindamycin

Concentration of clindamycin (%)	Zone of inhibition (mm)	Result
0.5	25.53	Strong activity
1	25.43	Strong activity
2	27.33	Strong activity
4	32.83	Strong activity

Table 8: Zone of inhibition of various cream formula bawang dayak

Various cream	Zone of inhibition (mm)	Result
Day 0		
F1	7.83	Weak activity
F2	9.53	Weak activity
F3	12.47	Moderate activity
F4	12.53	Moderate activity
Day 7		
F1	7.00	Weak activity
F2	8.20	Weak activity
F3	9.60	Weak activity
F4	9.83	Weak activity

for 24 h, and the zone of inhibition was measured [7] and recorded later on.

RESULTS AND DISCUSSION

Evaluation test of cream formula

Organoleptic appearance

The results of organoleptic test on day 0 showed that F1 had a lighter brown color than other formulas, it caused the concentration of ethanolic extract bawang dayak less concentration than other formulas. On day 7, all formulations became darker than before, this was probably due to ethanolic extract of bawang dayak content antioxidant compound. The odor of F3 and F4 on day 0 has sharper odor than F1 and F2, it caused concentration of ethanolic extract, but the odor has no change on the day 7 (Table 2 and Fig. 1).

Homogeneity observation

The observation of cream bawang dayak showed on day 0 all formulation homogen, but day 7, F3 and F4 showed separation phase between the oil phase and the water phase (Table 3).

PH observation

The pH observation showed all cream formula of bawang dayak around 6 on day 0 and day 7 (Table 6). The pH that suitable for topical application is same with pH of skin, between 4.5-6 [8].

Dispersion test

The dispersion test adding and without adding weight has an average of more than 3 cm to all creamy formula both on day 0 and day 7 (Table 5).

Adhesion test

Adhesion test of cream on day 0 was more than 4 s and time of adhesion decrease on day 7 (Table 4).

Antibacterial activity

The antibacterial activities 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) [9]. 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 stand until 7 days have decrease zone of inhibition in category weak activity (Table 8 and Figs. 2 and 3). Inhibition zone decrease on day 7 may be caused storage not in cool area like in refrigerator, so it was needed develop research further. This research showed that all formula with different concentration of ethanolic extract bawang dayak potential inhibit *P. acnes* that caused acne vulgaris although zone of inhibition smaller than clindamycin as positive control (Table 7 and Fig. 4).

Inhibition of all formula against *P. acnes* because ethanolic extract of bawang dayak contains secondary metabolites such as alkaloid, flavonoid, tannin, and saponin that can be potential has antibacterial effect against *P. acnes* [10]. Research about bawang dayak with the same type of plant specifically *E. bulbosa* parallel with research that conducted in India that stated bawang dayak has significant antibacterial activity [11].

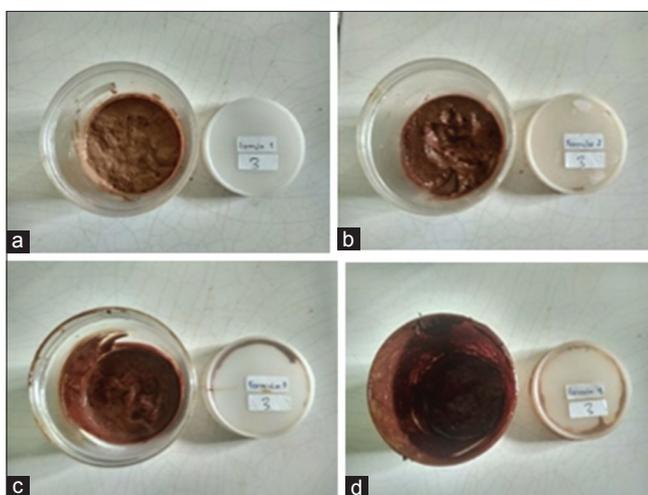


Fig. 1: Various cream formula ethanolic extract of bawang dayak; (a) F1, (b) F2, (c) F3, (d) F4

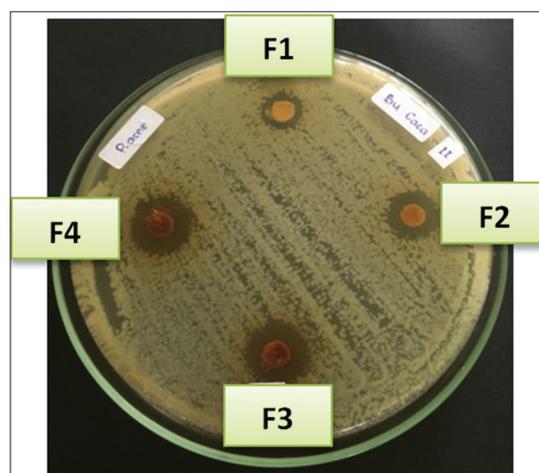


Fig. 3: Zone of inhibition all cream formula ethanolic extract of bawang dayak day 7

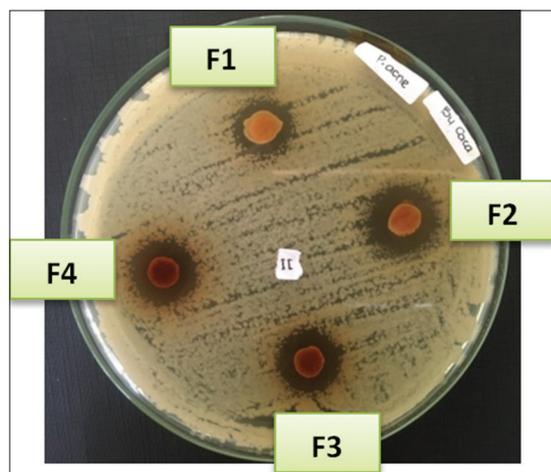


Fig. 2: Zone of inhibition all cream formula ethanolic extract of bawang dayak day 0

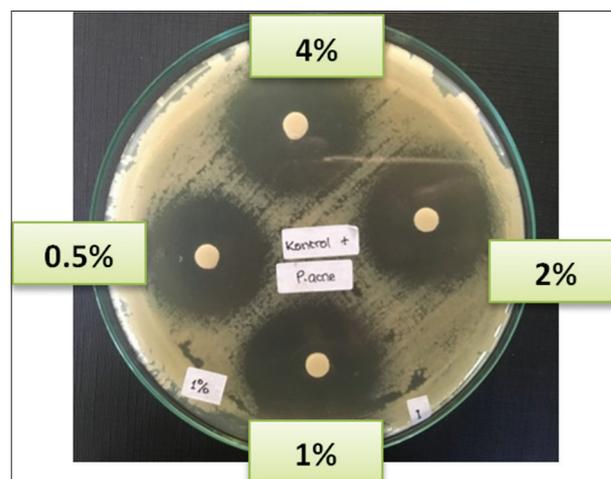


Fig. 4: Zone of inhibition clindamycin

CONCLUSION

Extract ethanolic of bawang dayak in all cream formula potential inhibits *P. acnes*, but inhibitory ability decreases on day 7. This research must be developed such as cream formula and improve effectiveness and combination with another material, so as the formula not separate and the effectivity is stable.

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