

ANTIBACTERIAL ACTIVITY TEST OF ACNE CREAM BAWANG DAYAK (*ELEUTHERINE BULBOSA* (MILL.) URB) AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Acne vulgaris is not dangerous disease but it can lead depression, acne is type of skin disease caused by bacteria. One of the causes of acne is *Staphylococcus aureus*. Based on research before, extract ethanol of bawang dayak from Central Kalimantan can inhibit *S.aureus*, so in this research ethanolic extract was made in cream formulation to improve the efficiency of using traditional medicine. The aims of this present study were to formulate anti acne cream consist ethanolic extract of bawang dayak and evaluate antibacterial activity of cream on day-0 and day-7 to see stability activity and preparation. The Methods of this study are cream formula of Bawang Dayak divided into 4 formulas with different concentration of ethanolic extract bawang dayak then was evaluated organoleptic characteristics, homogeneity, pH, adhesion test, dispersion test and in vitro antibacterial test against *S.aureus* with diffusion method. The results show the homogeneity of the cream shows F3 dan F4 separate and non homogen on day-7 but all formula pH suitable for topical application. Antibacterial activity of antiacne cream bawang dayak was indicated from zone of inhibition, on day-0 F1 = 11.50 ± 0.14 mm (weak activity), F2= 17.20 ± 0.85 mm (moderate activity), F3= 16.90 ± 0.28 mm (moderate activity) and F4= 17.45 ± 2.90mm (moderate activity) while on day-7 respectively F1= 10.25 ± 0.21 mm (weak activity), F2= 11.40 ± 0.28 mm (weak activity), F3= 13.35 ± 0.35 mm (moderate activity) and F4= 15.75 ± 1.20 mm (moderate activity), on day-7 zone of inhibition of all cream formula decrease but still can inhibit. So it can be concluded that all Cream Formula potential against *S. aureus* but this study must be improved both of preparation and stability activity.

Key words: Acne vulgaris, Bawang dayak, Cream, *Eleutherine bulbosa*, *Staphylococcus aureus*

INTRODUCTION

Nowdays people choose natural remedies for traditional cosmetics. Cosmetics are products which are used to purify and beautify the skin. The best reason for using traditional cosmetics because natural content in the herbs does not have any side effects in the human body (Mishra et al., 2014). Cosmetic products need to be effective and stable but also the acceptance by the consumer needs to be confirmed because the incorporation of natural extracts could confer undesirable characteristics, strong colors or aromas (Soto et al., 2018).

Empirically, Bawang dayak is known to have efficacy for treating skin diseases. One type of skin disease caused by bacteria is acne. Acne, a chronic inflammatory disease of the pilosebaceous units of the face, neck, chest and back, is the most common skin disorder occurring universally, with an estimated prevalence of 70-87%. It is a pleomorphic disorder characterized by both non-inflammatory (comedones) and inflammatory (papules, pustules, nodules) lesions (Kaymak and Onder, 2008). Bawang dayak contain chemical compounds like alkaloid, flavonoid, tanin, saponin and naphthoquinones which potential as antibacterial (Ardhany, 2019; Novaryatiin et al., 2019; Rani, 2018; Ravichandiran et al., 2019). Based on research Novaryatiin et al., 2019 ethanolic extract of bawang dayak (*Eleutherine bulbosa* (Mill.) Urb) positively can inhibit *Staphylococcus aureus*, so in this study ethanolic extract of bawang dayak was made in formula cream as a product of traditional cosmetic.

MATERIAL AND METHODS

Material

Bawang dayak (*Eleutherine bulbosa* (Mill.) Urb) were procured from farmer cultivation in Sei Gohong, Bukit Batu Palangka Raya, Central Kalimantan and identified at Indonesian Institute of Sciences Research Center For Biology. The part used is bulb of bawang dayak.

Extraction

The plant materials were washed with water and dried under the sun for 5-7 days. The dried plant materials were crushed by grinder. Then, the powder was extracted with 96% ethanol by using percolator and all extracts were evaporated by using rotary evaporator to reach a viscous extracts.

Phytochemicals Screening

The prepared cream of ethanolic extract of bawang dayak was subjected to phytochemical screening to detect the presence/ absence of secondary metabolites (Kaur and Prasad, 2016).

Formulation Preparation

The components of formulation were listed in Table 1. The components consist of oil soluble (stearic acid, adeps lanae, paraffin liquid) and water soluble (TEA, nipagin dan 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 phase and stir until homogen in mortar, oil phase add gradually, then stir until the cream base is formed. The last add oleum roses and stir ad homogen. The cream was evalutated on day-0 and day-7 without any special treatment.

Evaluation Test of Cream

- a. Organoleptic Properties
The cream was observed for color, odor and appearance
- b. 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 particles (Nazliniwaty et al., 2016)

c. pH Measurements

Determining pH of the preparation is done by using pH meter (El-Gied et al., 2015)

d. Dispersion Test

Cream with 0.5 g was placed in the middle of a round glass scale. Round glass which has been weighted placed there on and left for 5 minutes. After that followed with 50 g load, let stand for 1 minute and record the diameter of the spread cream, did the same thing with 100 g and 150 g (Safitri et al., 2016)

e. Adhesion Test

A total of 0.5 g of preparation was spread on the disc glass, on top of it other glass object placed and pinned under 1 kg load for 1 minute. 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 (Safitri et al., 2016)

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

Cream of ethanolic extract of Bawang Dayak were evaluated for in vitro antibacterial activity against acne vulgaris were caused by *Staphylococcus aureus* using the disc diffusion method with different concentrations of extract in cream formula.

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 38g in 1000 ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121°C for 15 minutes (Mhatre et al., 2014).

MHA plates were prepared and bacterial strains were inoculated by cotton swab and then antibiotic and cream with various concentration of extract bawang dayak applied in it. The plates were incubated at 37° C for 24 hours and the zone of inhibition was measured (Bhalodia and Shukla, 2011) and recorded later on.

RESULT AND DISCUSSION

A. Evaluation test of cream formula

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 (Fig 1.). Day-7 all formulations became darker than before, it was probably due to ethanolic extract of bawang dayak content antioxidant compound. The odor of F3 dan F4 on day-0 have sharper odor than F1 and F2 it caused concentration of ethanolic extract, but the odor has no change on the day-7. The types of all cream formula are oil in water (o/w).

The observation of cream bawang dayak on day-0 all formulation homogen, but on day-7 F3 and F4 showed separation phase between the oil phase and the water phase (Table 2.). The pH observation showed all cream formula of bawang dayak

around 6 on day-0 and day-7 (Table 3). The pH that suitable for topical application is between 4.5-6 same with pH of skin (Mali et al., 2015). The dispersion test adding and without adding weight has an average for more than 3 cm to all cream formula both on day-0 and day-7 (Table 4). Adhesion test of cream on day-0 was more than 4 second and time of adhesion decrease on day-7 (Table 5).

B. Antibacterial activity

Based on research before ethanolic extract of Bawang dayak can inhibit *Staphylococcus aureus* (Novaryatiin et al., 2019) as well as bawang dayak in cream in this study. The antibacterial activities can be classified into three levels: weak activity (< 12 mm), moderate activity (12-20 mm) and strong activity (> 20 mm) (Shahbazi, 2017). The inhibitory test results of control positive (clindamycin) shown on Table VI (Fig 2.), the antibacterial activity test of cream ethanolic extract bawang dayak on day-0 F1 have 11.50 mm inhibition zone (weak activity), F2 = 17.20 mm (moderate activity), F3 = 16.90 mm (moderate activity) and F4 = 17.45 mm (moderate activity), while all of the formulas that lasted for 7 days have decline of zone inhibition value but F3 and F4 still in same category, moderate activity (Table VII.) (Fig 3.). Inhibition zone decrease on day-7 may be caused not placed in special storage area such as a cold area in the refrigerator, so it was needed develop further study.

Inhibition of all formulas against *Staphylococcus aureus* because ethanolic extract of bawang dayak contain secondary metabolites like alkaloid, flavonoid, tannin and saponin. Many studies have suggested that flavonoid act in plants as antioxidant and antimicrobial, flavonoid is the largest group of phenol compounds that effectively inhibit the growth of viruses, bacteria and fungi (Harlita et al., 2018; Panche et al., 2016). The presence of alkaloid, saponin and tannin may serve as potential compounds that can act as antibacterial against bacteria acne causing like *Staphylococcus aureus* (Khan et al., 2018; Mabhiza et al., 2016; Min et al., 2008). Some of these bioactive compounds that are synthesized as secondary metabolites as the plant grows are also used to protect the plant against microbial attacks (Razmavar et al., 2014).

CONCLUSION

All cream formula of ethanolic extract bawang dayak potential inhibit *Staphylococcus aureus*, but on day-7 value of zone inhibition decrease. This study must be developed such as cream formula, improve effectiveness and combination with another material, so as the formula not separate and the effectivity is stable.

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TABLE AND FIGURE

Table 1. Various Cream Formula of Ethanolic Extract Bawang Dayak

Materials	F1	F2	F3	F4
Extract Ethanol of Bawang Dayak	5% (1250 mg)	10% (2500 mg)	15% (3750 mg)	20% (5000mg)
Ol. Rosae	12 gtt	12 gtt	12 gtt	12 gtt
Oily Phases:				
Stearic Acid	5000 mg	5000 mg	5000 mg	5000 mg
Adeps Lanae	750 mg	750 mg	750 mg	750 mg
Paraffin Liq	6250 mg	6250 mg	6250 mg	6250 mg
Aqueous Phase:				
Triethanolamin	375 mg	375 mg	375 mg	375 mg
Nipagin	25 mg	25 mg	25 mg	25 mg
Aquadest ad	25000 mg	25000 mg	25000 mg	25000 mg

Table 2. The 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 3. The 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 4. The 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 5. The 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

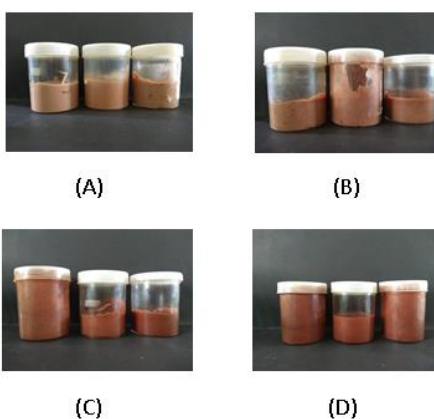


Figure 1. Various cream formula ethanolic extract of Bawang Dayak
 (A)= F1; (B) = F2; (C) = F3; (D) = F4

Table VI. Zone of Inhibition of Clindamycin

Concentration of Clindamycin	Zone of Inhibition (mm)	Result
0.5%	35.23 ± 1.55	Strong activity
1%	36.30 ± 1.31	Strong activity
2%	39.00 ± 2.43	Strong activity
4%	40.83 ± 3.10	Strong activity

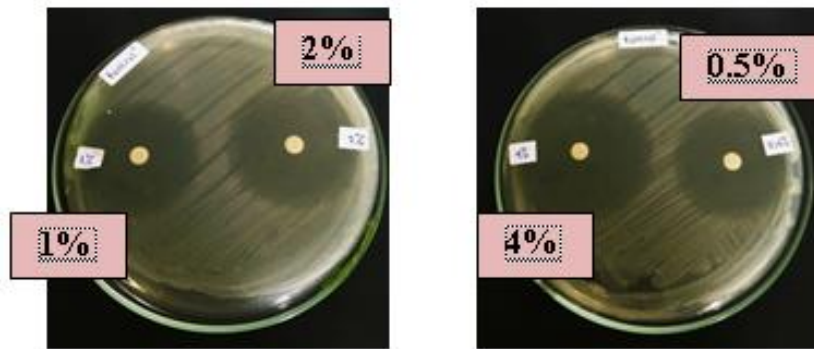


Figure 2. Zone of inhibition positive control, clindamycin

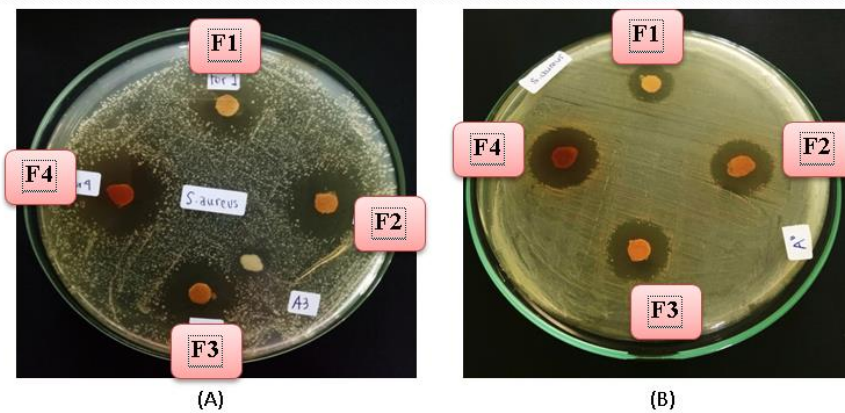


Figure 3. Zone of inhibition All Cream Formula Ethanolic Extract of Bawang Dayak Day-0 (A) and Day-7 (B)

Table VII. Zone of Inhibition of Various Cream Formula Bawang Dayak

Various Cream	Zone of Inhibition (mm)	Result
Day-0		
F1	11.50 ± 0.14	Weak activity
F2	17.20 ± 0.85	Moderate activity
F3	16.90 ± 0.28	Moderate activity
F4	17.45 ± 2.90	Moderate activity
Day-7		
F1	10.25 ± 0.21	Weak activity
F2	11.40 ± 0.28	Weak activity
F3	13.35 ± 0.35	Moderate activity
F4	15.75 ± 1.20	Moderate activity