RESEARCH ARTICLE

Formulation and Evaluation of Citronella Oil (*Cymbopogon nardus* (L.) Rendle) Cream for Acne Treatment

Tantri L. Nareswari*, Fidela O. Vrince, Erga Syafitri

Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, Lampung, Indonesia

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ABSTRACT

Acne is a prevalent skin disorder that affects 80–85% of teenagers globally. Citronella oil is one of the natural compounds that has been known to potentially treat acne, but its application is limited due to its greasiness and uncomfortable sensation on skin. This study aimed to optimize the concentration of cetyl alcohol as a viscosity enhancer that can produce a physically stable cream preparation and to evaluate the antibacterial activity of the optimized formula against *Propionibacterium acnes*. Following the emulsification process, the physical characteristics of the five cream formulas, including organoleptic, homogeneity, emulsion type, pH, adhesion, specific gravity, viscosity, and stability were evaluated. Formula 3 (F3), cream formulation with 6% cetyl alcohol having physical characteristics of a white homogeneous cream with a pronounced lemongrass scent, emulsion type o/w, pH of 6.30 ± 0.02, adhesion of 16.85 ± 0.58 s, a specific gravity of 1.031 ± 0.009 g/mL, and viscosity of 4418 ± 182 m Pas was chosen as the optimized formula. F3 was then subjected to antibacterial testing against *P. acne* and showed a 9.35 mm inhibition zone. Cream-based citronella oil, therefore, becomes a promising preparation for acne treatment.

Keywords: Cream, Emulsion, Citronella oil, *Propionibacterium acnes*, Viscosity enhancer.

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INTRODUCTION

Acne vulgaris, or acne, is a highly prevalent skin condition affecting 80–85% of adolescents worldwide.[1] Acne is a disease that affects which can be caused by excess sebum, the *Propionibacterium acnes* infection, keratinocyte shedding, hormones, genes (heredity), the environment, and medication or cosmetic use.[2,3] Acne typically develops on a place where it has a high density of sebaceous glands such as face, chest, upper back, and upper arms.[4] The sebum can be caught in the keratin and hardened. As a result of the trapped sebum, *P. acnes*, a member of the skin microflora, can grow and eventually produce comedones, inflammatory acne, papules, and even scar tissue.[3]

Acne therapy is necessary since it can have negative social effects, including depression, anxiety, and low self-esteem.[5] Topical acne medications such retinoids, benzoyl peroxide, azelaic acid, keratolytic, and alpha-hydroxy acids are commonly caused skin irritation or stinging, while the use of topical antibiotics in recent years has become increasingly ineffective due to the emergence of antibiotic-resistant strains.[6,7] Other alternatives that can provide relatively lower side effects are needed to treat acne. Natural ingredients are used more frequently as natural antibacterials since they have fewer side effects than conventional drugs.[8]

Lemongrass (*Cymbopogon nardus* (L.) Rendle), one of the many herbal plants from Indonesia, has been studied for its potential to combat acne-causing bacteria, including *P. acnes* and *Staphylococcus epidermidis in-vitro*.[9-10] Lemongrass contains citronella oil with citronellal, geraniol, and citronellol as active substances.[11] These compounds remove the intra-cellular hydrogen ions, causing immobility and eventually cell death in bacteria.[12] However, citronella oil cannot be applied directly to the skin since it may lead to irritation.[13] Topical dosage forms are favored for treating acne due to their ease of use.[14] Creams are semi-solid topical formulations in the form of a water-in-oil (w/w) or oil-in-water (o/w) emulsion.[15] Cream formulations have advantages such as the convenience of application, good spreadability, and release of active ingredients into the skin.[16]

Therefore, this research was aimed to optimize cetyl alcohol concentration as a viscosity enhancer in anti-acne cream preparations of citronella oil. Cetyl alcohol was selected as a thickener because it has emollient properties, an occlusive barrier on the skin, and helps to keep the skin hydrated.[17-18] The preparations were evaluated for their physical stability and the

*Author for Correspondence: tantri.nareswari@fa.itera.ac.id

optimum formula was then subjected to an *in-vitro* antibacterial test against *P. acnes*. This study is expected to produce stable and good-quality citronella oil cream compositions, effectively inhibiting the acne-causing bacteria *P. acnes*.

MATERIALS AND METHODS

Materials

The materials used in this study included citronella essential oil (PT. Rumah Atsiri Indonesia) derived from citronella plants (*C. nardus* (L.) Rendle) from Tawangmangu, Central Java. Tween 60, Span 60, cetyl alcohol, stearyl alcohol, sodium benzoate, glycerin, alpha-tocopherol, and aquadest are all pharmaceutical grade. Blood base agar and 0.9% NaCl was sterilized, and *P. acnes* ATCC 11827 was purchased from University of Indonesia.

Citronella Oil Cream Formulation

Optimization was carried out on cetyl alcohol as a viscosity enhancer with different concentrations (2, 4, 6, 8, 10%). Cream formulation was prepared by emulsification method as described by Lohani *et al.* with some modifications.[19] In a brief, the oil phase (1.6% span 60, cetyl alcohol, 2% stearyl alcohol, and 0.05% alpha-tocopherol) was mixed in a mechanical overhead stirrer at 100–200 rpm in the temperature of 60 ℃. The aqueous phase consisted of 3.4% tween 60, 3% glycerin, 0.2% sodium benzoate and water. The water phase was added to the oil phase at 100–200 rpm at 60˚C and mixed continuously until semi-solid consistency was achieved. The temperature is then lowered to <30 ℃. Citronella oil is gradually added until uniform.

The cream formed was then evaluated for its physical preparation, including organoleptic, homogeneity, emulsion type, pH, adhesion, specific gravity, viscosity, and stability test. The optimized formula was selected for an antibacterial activity test.

Organoleptic Test

Organoleptic evaluation of citronella oil cream was conducted by visual inspection of the formulation, including appearance, consistency, odor and color.[20]

Homogeneity Test

Testing the homogeneity of citronella oil cream preparations was carried out by taking 1-gm of cream at the top, middle and bottom, then smearing it on an object glass. Cream preparations can be said to be homogeneous if there are no visually visible coarse grains.[21]

Emulsion Type Test

The type of emulsion is determined by adding water on a certain amount of cream. The cream that can be diluted is the O/W type, whereas the cream that cannot be diluted is the W/O type.[22]

pH test

Sample was weighed as much as 0.5 gm, dissolved in 50 mL of distilled water, and tested with a pH-meter.

Adhesion Test

The adhesion test was carried out by attaching sufficient cream to two glass objects, then pressing it with a load of 0.5 kg and then adding a load weighing 20 gm for 5 minutes. Furthermore, the time when the cream was released from the two glass objects was recorded.[23]

Specific Gravity Test

The specific gravity test was carried out using a pycnometer.[24] A 25 mL empty and clean pycnometer is weighed first, then 25 mL of cream is added and closed, then weighed. Do the same for the water as a control. Determination of specific gravity with the formula (1).

$$\rho 2 = \frac{w2-w0}{w1-w0} x\ \rho 1 \qquad (1)$$

where ρ1 = specific gravity of water; ρ2 = specific gravity of cream; W2 = pycnometer with cream; W1 = pycnometer with water; W0 = empty pycnometer.

Viscosity Test

Viscosity testing was carried out using a viscometer germany Rotavisc HiVi I (Ika) using spindle number 10 at 100 rpm. Three replications of samples were measured.

Stability Study

The five formulations were subjected to a room temperature stability test for 28 days and three freeze-thaw cycles. This test was carried out at room temperature 25˚C days 0, 1, 7, 14, 21, 28. Freeze-thaw storage temperature was -18˚C for 24 hours and 25˚C for 24 hours for 1 cycle.[25] The physical properties (organoleptic, homogeneity, adhesion, pH, and viscosity) were evaluated at each time point.

Optimized Formula Determination

The optimized formula derived from the physical tests (organoleptic, adhesion, homogeneity, pH, and viscosity) which met the requirements at day 0. Subsequently, the optimized formula was subjected to antibacterial activity against *P. acnes*.

Antibacterial Activity Test

P. acnes were tested for antibacterial activity using agar diffusion method anaerobically as described by Jusuf *et al.*[26] The materials and tools were sterilized before use. Blood base agar was made by dissolving 10 grams of media in 250 mL of distilled water. The media was then sterilized for 15 minutes at 121°C and poured into a petri dish once the temperature had dropped to 40–45°C. *P. acne* suspension in 5 mL was subsequently added into the petri dish and the turbidity was assessed using 0.5 McFarland. Following the inoculation of *P. acnes* bacteria into the media, four wells were prepared in each petri dish using a sterile tip. The samples were erythromycin, citronella-scented oil, sodium benzoate, optimum cream formula, and distilled water. The plates were then incubated at 37°C for 24 hours. Replication was carried out three times. Observations were conducted by measuring the inhibition zone which was formed.

Data Analysis

Data on organoleptic test results, pH, cream type, emulsion height, specific gravity and homogeneity were analyzed descriptively. While the results of the adhesion and viscosity test at day 0, and the antibacterial test were analyzed using one-way anova method with a 95% confidence level (SPSS version 26).

RESULTS AND DISCUSSION

Citronella oil contains citronellal, citronellol, and geraniol compounds that act as antibacterial agents.[9-11] Nevertheless, citronella oil cannot be used directly since it might irritate.[13] As a result, in this study citronella oil was formulated in cream dosage form.

Cream is a suitable dose form for hydrophobic compounds since it comprises water and oil phase.[15] The required oil HLB must first be optimized to identify the surfactant accounts for the most stable citronella oil cream. In this study, we used the combination of span 60 and tween 60 as emulsifiers. The creaming index determines the concentration of tween and span is close to 1, where the change of emulsion height was minimum.[27] The surfactant concentrations for tween 60 was 3.4% and for span 60 was 1.6% since they had the highest creaming index, 0.93 (data not shown). The ratio value was selected since less creaming will form in the emulsion as the ratio closes to 1.

Furthermore, optimization of cetyl alcohol as a viscosity enhancer was performed at five different formulas with cetyl alcohol concentrations ranging from 2 to 10%. The five formulas underwent emulsion type, pH, homogeneity, adhesion, specific gravity, viscosity, and stability test.

Figure 1 displays the organoleptic effects of citronella oil cream. The citronella oil cream formed in this study had a white and semi-solid cream with a distinctive odor. No organoleptic changes or separation were observed during the 28-day storage at room temperature 25°C and freeze-thaw test in all of the five formulas. The findings hence indicated that five cream formulas were stable throughout the storage.

Cream is homogeneous when no obvious coarse granules are visible.[21] The homogeneity test revealed that the five formulations were all homogeneous. Phase separation was not observed during 28-day storage at room temperature (25°C) and freeze-thaw at room temperature.

The five cream formulas can be diluted with water, indicating that they are an o/w (oil in water) emulsion type. Cream with O/W emulsion-type is favored since it is water-rinsable and does not leave a greasy feeling.[28]

Figure 2 shows the result of the pH test. The physiological pH value of the skin ranges from 4.5-6.5.[29] The pH test revealed that formulas 3, 4, and 5 fulfilled the skin pH, but formulas 1 and 2 had higher pH values 6.6–6.8. The pH was increased at lower cetyl alcohol concentration. This result agrees with Handayani *et al.,* which found that the pH value will decrease along with the increase in cetyl alcohol content.[30] The five formulas proved stable because storage under ambient

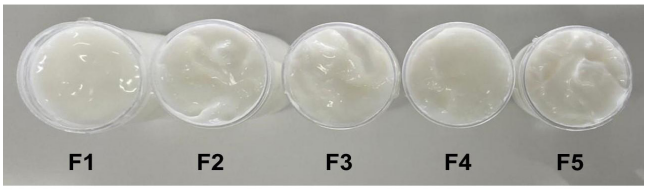

Figure 1: Organoleptic results of citronella oil cream. Citronella oil with cetyl alcohol 2% (F1); 4% (F2); 6% (F3); 8% (F4); 10% (F5).

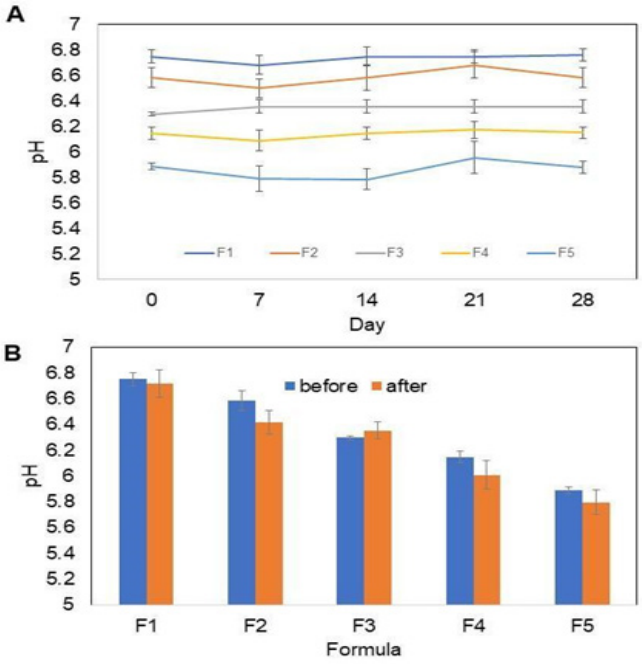

Figure 2: pH results of citronella oil cream at room temperature storage (A) and freeze-thaw (B) stability test. Citronella oil with cetyl alcohol 2% (F1); 4% (F2); 6% (F3); 8% (F4); 10% (F5).

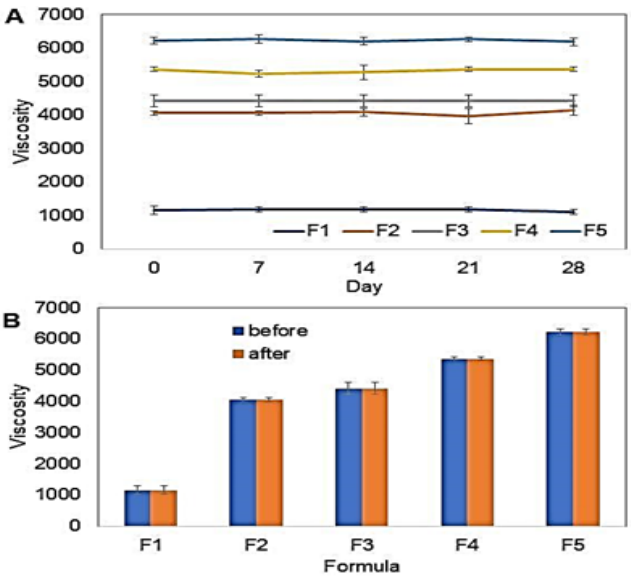

Figure 3: Viscosity results of citronella oil cream at room temperature storage (A) and freeze-thaw (B) stability test. Citronella oil with cetyl alcohol 2% (F1); 4% (F2); 6% (F3); 8% (F4); 10% (F5).

conditions and freeze-thaw cycles revealed no significant pH changes.

Figure 3 displays the viscosity test results. A good cream viscosity was between 2,000 and 50,000 mPas.[31] The viscosity values of the five formulas were in the ranges of 2798–6364 mPas for formulas 2, 3, 4, and 5, whereas formula 1 did not meet good cream requirements since the values were 1011–1287 mPas. The viscosity of cream increases significantly with the increase of cetyl alcohol concentration (from F1 to F5). This result agrees with Kang *et al.* and is consistent with the nature of cetyl alcohol as a viscosity enhancer.[32]

The viscosity result is in agreement with the cream-specific gravity result, where the increase of cetyl alcohol concentration increased the specific gravity. Formula 1 has the lowest specific gravity (0.973 $\pm$ 0.009 g/mL), while formulas 2, 3, 4, and 5 increased accordingly (1.006 $\pm$ 0.004, 1.031 $\pm$ 0.009, 1.055 $\pm$ 0.006, and 1.082 $\pm$ 0.007 g/mL, respectively). Previous study found that a good cream specific gravity ranging from 0.95 to 1.05 g/mL,[31] hence the formulas 1, 2, 3, and 4 met the criteria. Statistics analysis reveals that there are no notable specific gravity changes upon room temperature storage.

The subsequent test is an adhesion test. The adhesive result of five formulations increases significantly ($p < 0.05$) with the increase of cetyl alcohol, as shown in Figure 4. This result agrees with Kulawik-Pióro *et al.* which mentioned that cohesiveness and index of viscosity were related to the cream adhesiveness.[33] The adhesive requirement for a cream preparation is not less than 4 seconds, hence five cream formulas fulfilled the acceptance criteria of good cream.[34]

The optimum preparation will be chosen for antibacterial testing by analyzing the physical parameters during room temperature storage and freeze-thaw. Formula 3 was chosen for the antibacterial test out of all the formulas tested because it produced the best test results, where formula 3 fulfils all test requirements for acceptable physical properties of cream preparations.

For the antibacterial test, the average result of the inhibition zone against *P. acnes* for cream formula 3 was 9.35 mm, citronella oil was 29.22 mm, and erythromycin was 27.33 mm. The aquadest, sodium benzoate, and cream base did not produce any inhibition zone, indicating that the components did not affect the optimum cream formula inhibition zone and that the formula 3 inhibition zone are proven to come from the active substance.

Compared to citronella oil cream in optimum formula (formula 3), the results of erythromycin and citronella oil inhibitory zones were higher. This result is in accordance with El-Gied *et al.*[35] Cunha *et al.* mentioned that antimicrobial agents (such as thymol) bind to tween and reduce their antibacterial activity.[36] The active component of essential oils usually dissolved in hydrophobic bases, thereby preventing their release due to their strong affinity for the lipophilic components (semi-solid formulation coefficient partitions).[37-38]

Based on the results of the investigation, formula 3 with a concentration of 6% cetyl alcohol is the optimum cream formulation and has been shown to have antibacterial action against the *P. acnes*.

CONCLUSION

The optimum concentration of cetyl alcohol that produces the best cream preparation is 6%. Citronella oil cream preparations possess potential antibacterial activity against the acne-causing bacteria, *P. acnes*.

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