

ANTI-ACNE (*Acne Vulgaris*) EMULGEL FORMULATION of AROMATIC OIL From PATCHOULILEAVES (*Pogostemoncablinbenth*) Andin VITRO ANTIBACTERIAL ACTIVITY TEST Against *propionibacterium Acnes*

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Abstract

Patchouli leaves (*Pogostemon cablin* Benth) aromatic oil contains *alcoholic patchouli* which has antibacterial activity. This research aim is to get an effective, safe, and stable anti-acne emulgel preparation from patchouli leaves (*Pogostemon cablin* Benth) aromatic oil especially during storage time. Concentration of patchouli leaves (*Pogostemon cablin* Benth) aromatic oil used in the anti-acne emulgel preparation is 0.2%; 0.3%; and 0.4% (v/v). Stages of research that has been done are materials collection, antibacterial activity test of aromatic oil, determination of minimum inhibitory concentration, emulgel basis selection, emulgel formulation, physical stability test including organoleptic checking, viscosity and pH measurement, antibacterial preparation activity test for 30 days of storage times and preparation safety test. Results from the research showed that all of the anti-acne emulgel preparations had good quality and did not trigger any inflammation or irritation on the user skin.

Keywords: Patchouli leaves, aromatic oil, emulgel preparation, anti-acne

1. Introduction

Acne is a common disease that can be found on human skins, marked with *seborrhea* (reddish scaly skin), blackheads, pinheads, pimples, nodules, and sometimes tissue graters (Stein Gold, 2013). Severe acne is considerable as inflammation, but acne can also exist as non-inflammation forms (Joyce, 2013).

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Main factors causing acne were hormonal instability, affecting foods, stress and lacks of skin hygiene which in the end can generate overproduction of sebum, follicles blockage, and acne-causing bacterial (*Propionibacterium acnes* and *Staphylococcus epidermidis*) infection and colonization (Dhillon, 2013).

Propionibacterium acnes apilosebaceous gland normal flora on human skin. The mechanism of acne formation caused by bacteria was bacteria produced lipases that breakdown free fatty acids from skin lipids (Lyte, 2009). This fatty acid resulted in tissue inflammation when interacted with immune system and promoted acne formation (Sanjay, 2009).

One of the natural antibacterials that had function as anti-acne was patchouli leaves aromatic oil (*Pogostemon cablin*, Benth). Patchouli aromatic oil contains alcohol, eugenol, benzaldehyde, cinnamic aldehyde, cariophylen, α -patchaolena, and bulnessen (Kumara, 2011). Based on previous research, patchouli leaves aromatic oil (*Pogostemon cablin* Benth) was effective against *Staphylococcus epidermidis* and *Propionibacterium acnes*. To increase effectivity of aromatic oil usage on skin the formulation of aromatic oil in emulgel form was done.

Emulgel form is a gel consist of lipid phase dispersed in water and is a two-phase system which contains water and lipid molecules. Emulsion that contains gelling agents has stronger gel consistency, has lower risk of coalescence formation and also stabilized viscosity, so that can reduce foamy structure of the emulgel itself.

In this research, patchouli leaves (*Pogostemon cablin* Benth) aromatic oils was formulated as emulgels which has activity as an effective anti-acne substance against *Propionibacterium acne* and predicted to be safe and stable during storage time.

Materials and Method

Equipments

Incubator (Sakura IF-4), autoclave (Hirayama), petri discs (Pyrex), volumetric micropipettes (Eppendorf), aluminium perforators, homogenizer, digital pH meter (Nethorm®), analytical scaler (Mettler Toledo), viscometer (Brookfield), and other equipment that is commonly used in Pharmaceutical Microbiology Laboratory.

Materials

HV-505Aquepec, triethanolamines (Bratachem), propylene glycols (Bratachem), Tween 80 (Bratachem), patchouli leaves aromatic oil, and aquadest.

Research Method

1. Materials Collection

Patchouli leaves (*Pogostemoncablin* Benth) aromatic oil was collected from the Faculty of Agricultural Technology and Sciences, Padjadjaran University, Indonesia.

2. Antibacterial activity test of patchouli leaves aromatic oil

Antibacterial activity test of patchouli leaves aromatic oil was performed by using various concentrations in agar diffusion method with perforation technique. Bacterial suspensions were put into 20 μ L petri dish, then 20 ml of agar medium was added and shaken gently in order to make bacterial suspension and the media homogeneously solidified. After the agar medium containing bacterial suspension has been solidified, then media were perforated by using appropriate perforator to make holes, and in the end patchouli leaf aromatic oil were injected into those holes with various concentrations. After it was incubated for 18-24 hours at 37 °C, diameter of inhibition formed were observed.

3. Determination of Minimum Inhibitory Concentration

Determination of MIC were performed by using scratch method. Aromatic oil of patchouli leaves were mixed with liquid nutrient agar in a sterile petri dish using a certain ratio. Petri dish were shaken until the mixture becomes homogeneous, allowed to solidify at room temperature, then streaking the bacterial suspension test using a wire loop. All petri dishes that were scratched with test bacteria were incubated at 37 °C for 18-24 hours.

4. Emulgel basis orientation

Basis selection was done by using four basis gel. The first basis (B1) contains 0,25% aquapec HV-505; 0,375% TEA; 1,25% propilenglikol; and added with aquadest to 100 mL. Second basis (B2) contains 0,5% aquapec HV-505; 0,75% TEA; 2,5% propilenglikol; and added with aquadest to 100 mL. Third basis (B3) contains 0,75% aquapec HV-505; 1,175% TEA; 3,75% propilenglikol; and added with aquadest to 100 mL. Fourth basis (B4) contains 1% aquapec HV-505; 1,5% TEA; 5% propilenglikol; and added with aquadest to 100 mL.

5. Emulgel formulations by using various concentration of Patchouli Leaves Aromatic Oil

Selected gel base formulas were added with patchouli leaf aromatic oil emulsions by using various concentrations. In this research three variations of emulgel formulation will be used. The first one contains 0,2% of patchouli leaves aromatic oil and 0,01% of tween 80. The second one contains of 0,3% patchouli leaves aromatic oil and 0,015% of tween 80. The third one contains of 0,4% patchouli leaves aromatic oil and 0,02% of tween 80.

6. Physical stability test of Anti Acne Emulgel Formulation

Anti-acne emulgel that has been made is stored for 30 days at room temperature ($\pm 25^\circ \text{C}$) and physical changes were observed, including organoleptic examination, viscosity, and pH measurements.

7. Anti-Acne Emulgel Antibacterial Activity Tests

Antibacterial activity test against *Propionibacterium acne* bacteria were done by using agar diffusion method with perforation technique.

8. Safety test of anti-acne emulgel

Safety test of the emulgel were performed by using patch test methods to 10 volunteers.

9. Data Analysis

Data analyses were performed by using Duncan method.

Result and Discussion

1. Materials collection results

Patchouli leaves aromatic oil were obtained from Faculty of Agricultural Science and Technology.

2. Antibacterial activity test of patchouli leaves aromatic oil result

The result of antibacterial activity test of patchouli leaves aromatic oil shows that this oil has antibacterial activity against *Propionibacterium acne*.

3. Determination of MIC result

The MIC determination of patchouli leaves aromatic oil shows that from the five variations of test concentration that was used, which is 0,06; 0,07; 0,08; 0,09; and 0,1% (gram/mL) minimum inhibitory concentration lies between concentration of 0,09 – 0,1%.

4. Basis orientation result

Basis orientation results were shown that B2, B3, and B4 has more viscose consistency than B1 and considered not too good to be used as emulgel basis. Based on the observation result, thus the basis chosen to be used was B1.

5. Anti-acne emulgel formulation result

The result of anti-acne emulgel formulation with various concentration of aromatic oil shows that gel without aromatic oil has more viscosity, colourless, and odourless. Gel with aromatic oil of 0,2%; 0,3%; and 0,4% concentration has less viscosity, white color and typical odor.

6. Physical stability test of anti-acne emulgel from patchouli leaves aromatic oil

Various concentration of aromatic oil in anti-acne emulgel that has been made were stored for 30 days in room temperature ($\pm 25^{\circ}\text{C}$) and then physical properties were observed including organoleptic observation, viscosity changes, and pH measurement.

- A. The result of organoleptic observation shows that during storage time, emulgel does not show any changes in odor and color.
- B. The result of viscosity measurement during storage time was shown on Figure 1.

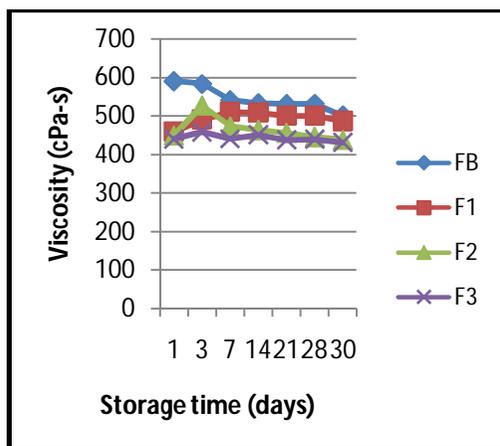


Figure 1. Viscosity graphic of anti-acne emulgel

Meaning:

- FB : gel without aromatic oil
- F1 : gel with aromatic oil (0.2%)
- F2 : gel with aromatic oil (0.3%)
- F3 : gel with aromatic oil (0.4%)

From the graphic shown we can assume that the bigger concentration of aromatic oil cause decreasing of viscosity. The gel that was combined with patchouli leaves aromatic oil has smaller viscosity value compared to gel without aromatic oil.

During storage time viscosity tends to decrease. This was because there were any affection of temperature and pH changes.

Statistical analysis result shows that there were no viscosity differences that occur during storage time.

C. pH measurement result of anti-acne emulgel during storage time were shown on Figure 2.

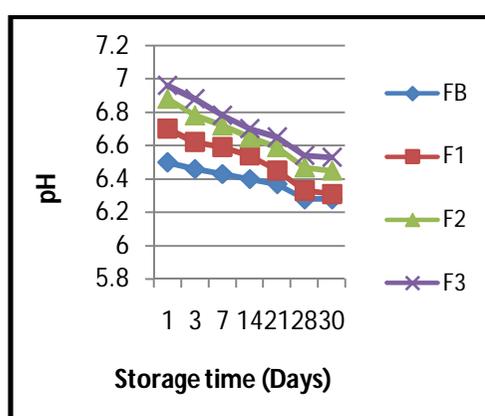


Figure 2: pH measurement graphic during storage time

Meaning:

- FB : gel without aromatic oil
- F1 : gel with aromatic oil (0.2%)
- F2 : gel with aromatic oil (0.3%)
- F3 : gel with aromatic oil (0.4%)

From the figure shown we can understand that emulgel level of pH were decreasing during 30 days of storage time. Thus we can barely conclude that the pH of anti-acne emulgels tends to be acidic. This was because the aromatic oil that were added into the emulgel has acid property shown by its pH that has value on 3.9. Despite of that, the pH of emulgel preparation that has been made were still on safe level for skin topical requirements which was at the range 4-8 (Aulton, 1988). Statistical analysis shows that there was significant pH changes during storage time.

7. Safety test result

The result of safety test shows that there were no skin irritation that occur by using FB, F1, F2, and F3 preparation. Thus, we can conclude that the anti-acne emulgel is safe to be used.

Conclusion and Suggestion

Conclusion:

This research shows that aromatic oil from patchouli leaves can inhibit the growth of *Propionibacterium acne* bacteria so that it can prevent the occurrences of acne in skin. And then from this research we can also know that emulgel preparation that has been made fulfill the pharmaceutical requirement and stable during storage time. Statistical analysis shows that there was no effect of storage time to the viscosity value changes, but there was significant changes of the viscosity caused by the pH. Based on skin irritation test that has been performed, the emulgel that has been made is safe to be used.

Suggestion:

From this research, we strongly recommended next researcher to fix the odor problem of the emulgel made, this can be done by using *corrigendodoristo* make this emulgel more esthetically attractive.

References

- Dhillon, K.S. 2013. *Study of microbiological spectrum in Acne vulgaris: an in vitro study*. Scholars journal of applied medical sciences (SJAMS); 1 (6): 724-727.
- Joyce, R. 2013. *The role of inflammation in Acne vulgaris: multiple lines of evidence support a pivotal role for inflammation in the pathogenesis of Acne vulgaris*. Cover focus. 22-24.
- Kumara, S. 2011. *Analysis of genetic variability in Patchouli cultivars by using RAPD markers*. Research in Biotechnology, 2(6): 67-71.
- Lyte, P. 2009. *Heat killed Propionibacterium acnes is capable of inducing inflammatory responses in skin*. ExpDerm; 18: 1070-1072.
- Sanjay, B. 2009. *Pathogenesis of acne vulgaris: recent advances*. Journal of drugs in dermatology.
- Stein Gold, L.F. 2013. *What's new in acne and inflammation*. J Drugs Dermatol 12(6): s67-s69.