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Effectiveness Test Of Anti-Acne Emulsigel Preparation Based On Clove Leaf Oil (Syzygium Aromaticum (L.) Merr & Perry)

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Abstract –Acne is a common skin problem caused by the accumulation of oil that blocks the hair follicles. Factors contributing to acne include bacterial colonization by Staphylococcus epidermidis and Propionibacterium acnes, increased sebum production, and follicle disturbances. Generally, acne treatment aims to reduce sebum production, address inflammation, decrease bacterial colonization, and improve skin conditions. The commonly used therapies include antibiotics and retinoids, although challenges such as antibiotic resistance and side effects exist. As an alternative, clove (Syzygium aromaticum) contains eugenol, which has antibacterial properties that are effective against the growth of Propionibacterium acnes. A combination of emulsion and gel (emulsisponge) can be a stable and effective formulation for acne treatment. This study aimed to formulate clove leaf oil into an emulsified gel preparation, evaluate the formulation, and test its antibacterial activity. The study began with distillation of clove leaf oil and its formulation into an emulsion gel. Evaluation of the emulsion included organoleptic, homogeneity, pH, viscosity, particle size, skin irritation, and antibacterial activity tests. The results showed that The emulsified gel was white, aromatic, thick, and homogeneous. The emulsion has pH (4.5-6.5), viscosity (6500-9500 mPa.s), and particle size (8.3-14.1 µm), and remains stable for 12 weeks at room temperature, low temperature, and high temperature, without causing skin irritation. Antibacterial test results indicated that the emulsion exhibited a highly active inhibition zone.

Keywords: emulsiongel; clove leaf oil; antibacterial; anti-acne

1. INTRODUCTION

The skin is the largest organ covering the entire human body and is located in the outermost layer. One skin problem often experienced by people, especially teenagers, is acne. In the medical world, acne is known as acne vulgaris. This condition occurs because of the accumulation of oil glands under the skin that clog hair follicles, thus pushing out fat that forms acne(Fitri et al., 2023). The causative factors of acne include bacterial colonization of the sebaceous glands, increased sebum production, changes in its composition, hyperpigmentation of polysebaceous follicles, and disruption of keratinocytes in the upper follicles.

One of the main bacteria causing acne is Staphylococcus epidermidis and*Propionibacterium acnes* (Silvyana et al., 2024). These bacteria can trigger inflammation and abscess formation, especially in conditions where pores are clogged due to excess sebum production. By producing lipase, Staphylococcus epidermidis converts triglycerides in the sebaceous glands into free fatty acids that can cause skin infections. B. acnes bacteria are the main microorganisms found in the infrainfundibular area and can reach the skin surface by following sebum flow.(Warsa. U. C, 1994).

Acne treatment generally aims to reduce sebum production, inflammation, repair follicle abnormalities, reduce bacterial colonization, and improve skin appearance. Commonly used

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therapies include topical preparations and systemic medications such as antibiotics and retinoids. However, problems such as antibiotic resistance, retinoid side effects, and drug allergies pose challenges in dermatological treatment. Therefore, various studies have explored the use of medicinal plants as alternative therapies.(Dasawanti et al., 2022)

Cloves (Syzygium aromaticum), which belong to the Myrtaceae family, are a spice plant native to Indonesia. In addition to being used in the food, beverage, medicine, and cigarette industries, cloves have a high nutritional content and are rich in essential oils, resins, tannins, and other active compounds.(Nafisah Isnawati & Pujiastuti, 2024). Eugenol, an aromatic compound with antibacterial properties, is the main component of clove EO.(Intaningtyas et al., 2023). The essential oil of clove leaves contains up to 98% eugenol.(Guenther, 1990). Lova et al. (2018) showed that clove leaf oil at a concentration of 20% can inhibit the growth of Propionibacterium acnes bacteria with an inhibition zone diameter of 18.58 mm.

An emulsion is a liquid preparation containing active ingredients dispersed in a carrier liquid with stabilization using an emulsifying agent or surfactant. To avoid phase separation, an emulsifying agent was used to maintain the stability of dispersed liquid droplets.(Ministry of Health of the Republic of Indonesia, 1995). Gels, on the other hand, are semi-solid liquid-based preparations with structures that can be transparent or opaque and are usually used for external applications. The combination of emulsions and gels, known as emulsion gels, offers new, stable, and effective dosage forms.(Devi Suman et al., 2020). Therefore, researchers are interested in developing emulsigel preparations based on natural ingredients, such as clove leaf essential oil, as an alternative acne treatment that is safer and more effective for public use.

2. RESEARCH METHODS

Tools and materials

The tools and materials used in this study included a thermometer, Erlenmeyer flask (Pyrex), autoclave (Express Equipment), incubator (Memmert), laminar airflow cabinet (Astec HLF I200 L), micropipette (Eppendorf), caliper (Electric Digital Caliper), paper disk, petri dish (Iwaki), viscometer (NDJ-8S), stirring rod (Rofa), particle size analyzer (Horiba SZ-100 and Fristch), centrifuge (Hitachi), vortex (Biosan), analytical balance (Ohaus Pioneer), Du Nuoy tensiometer, and magnetic stirrer (E-Liquid). The materials used included clove leaf oil, nutrient agar medium (Oxoid), nutrient broth agar medium (Oxoid), dimethyl sulfoxide (Merck), KOH (Merck), Na2SO4 (Merck), and triethanolamine (CV. Rudang Jaya), and Carbopol 940 (CV. Rudang Jaya), and Tween 80 (CV. Rudang Jaya), and methyl paraben (CV. Rudang Jaya) and propylparaben (CV. Rudang Jaya), and distilled water (CV. Rudang Jaya),

and Staphylococcus epidermidis were obtained from the Biology Laboratory of the Faculty of Pharmacy, University of North Sumatra.

Clove Leaf Oil Distillation

The clove leaf simplicia was placed in a distillation flask, and distilled water was added. Distillation was performed for 8 h. The oil layer that was formed was then treated with anhydrous Na2SO4 to absorb the remaining water. The process was performed overnight. After that, the clove leaf oil was collected and placed in a dark colored vial.(Lova et al., 2018).

Emulsigel Preparation Formulation

The emulsion was prepared by dissolving methyl paraben and propylparaben in heated distilled water. Tween 80 was added and the mixture was homogenized at 70°C (water phase). The clove leaf oil and PEG 400 were homogenized at 70°C. After both phases were ready, the oil phase was slowly added to the water phase and homogenized until the emulsion reached room temperature. Finally, the emulsion that was formed was added to the gel base and stirred until a white gel emulsion was prepared with the distinctive aroma of clove leaf oil. The emulsion gel preparation formula is shown in Table 1. and Table 2.

Table 1. Gel Preparation Formula

Gel Material	Concentration
Carbopol 940	2
Triethanolamine	1
Aquadest ad	100

Table 2. Emulsigel Preparation Formula

Emulsion Material	Concentration
Clove Leaf Oil	6
Tweens 80	0.5
PEG 400	2.5
Methyl Paraben	0.1
Propyl Paraben	0.02
Gel base	20
Aquadest ad	80

Clove Leaf Oil Emulsigel Evaluation

- **Organoleptic Test**

Organoleptic tests were conducted to assess the physical appearance of the emulsigel preparation by considering several aspects, namely, the shape, smell, and color of the preparation. This evaluation is important to ensure that the product has appropriate visual quality and is comfortable to use.(Kresnawati et al., 2022).

- **Homogeneity Test**

Homogeneity test was conducted to ensure that the active ingredients were evenly distributed. A total of 50 mg of the emulsion gel preparation was applied to a glass object, and coarse grains were observed. If there were no coarse grains, the preparation was considered homogeneous.(Devi Suman et al., 2020)

- **pH Test**

A pH test was carried out to ensure the acidity level of the preparation so as not to cause irritation to the skin. This test also aims to ensure that the pH of the preparation is in accordance with the SNI 16-3499-1996 standard, which is between 4.5 and 8 for skin pH.(Chandra & Rahmah, 2022).

- **Viscosity Test**

Viscosity testing was performed by inserting the preparation into a beaker glass and selecting an appropriate spindle number. An NDJ-8S viscometer was used to measure the viscosity of the preparation.

- **Emulsion Type Test**

The emulsion-type test was performed by adding methylene blue to the preparation, and then applying it thinly to a glass object. In the M/A type, the water surrounding the oil phase is blue.(Murdiana et al., 2022)

- **Particle Size Test**

Emulsification gel particle size testing using the Fritsch particle size analyzer.

- **Stability Test**

Low temperature storage stability tests were conducted at room temperature (28±2°C), low temperature (4±2°C), and high temperature (40±2°C), for 12 weeks, then every 2 weeks, the formulation was visually inspected for any changes in physical condition, color, odor and shape (Jufri et al., 2017).

- **Skin Irritation Test**

An irritation test was conducted on the emulsigel preparation to determine whether the preparation could cause skin irritation. The irritation test was conducted on 12 panelists using the patch test method, in which the preparation was applied to the skin behind the panelist's ear for 24 h. Irritation is divided into two categories: primary irritation, which appears immediately after contact with the skin, and secondary irritation, which occurs several hours after contact(Directorate General of POM, 1985).

Antibacterial Activity Test of Emulsigel Preparations

Antibacterial activity testing was performed on emulsion gel preparations using the agar diffusion method. 0.1 mL of bacterial inoculum was put into a petri dish, then 15 mL of sterile nutrient agar media that had been dissolved and cooled to a temperature of 45°C was added. After that, the media and bacterial suspensions were homogenized on the table surface (laminar airflow cabinet). A backup paper that had been soaked in the test solution for ±15 min was placed on the solidified media. The Petri dishes were then incubated at 35±2°C for 24 h. The diameter of the inhibition zone was measured using a digital caliper and the test was repeated three times (in triplicate).

3. RESULTS AND DISCUSSION

Evaluation of Emulsigel Preparations

- **Organoleptic Test Results**

The emulsigel preparation had a white color with a distinctive aroma of clove leaf oil. In addition, this preparation showed good stability because there was no phase separation, which means that the oil and water phases in the emulsigel were evenly distributed.

- **Homogeneity Results**

The homogeneity results of the clove leaf oil emulsion gel preparation showed that no coarse grains were found on a piece of glass; therefore, it can be concluded that the emulsion gel preparation was homogeneous. The purpose of homogeneity testing was to ensure that the preparations had an even distribution of the drug ingredients. A homogeneous preparation will provide optimal results because the drug ingredients are evenly dispersed so that each part of the preparation contains the same amount of drug ingredients. If drug ingredients are not properly dispersed, the drug will not provide the desired therapeutic effect.(Susilowati & Wahyuningsih, 2014).

- **pH Test Results**

The pH measurement was carried out using a pH meter to ensure that the pH of the preparation was in accordance with the skin pH, which was 4.5–8.5, so that it was safe and did not cause irritation. The results of the pH tests are shown in Figure 1.

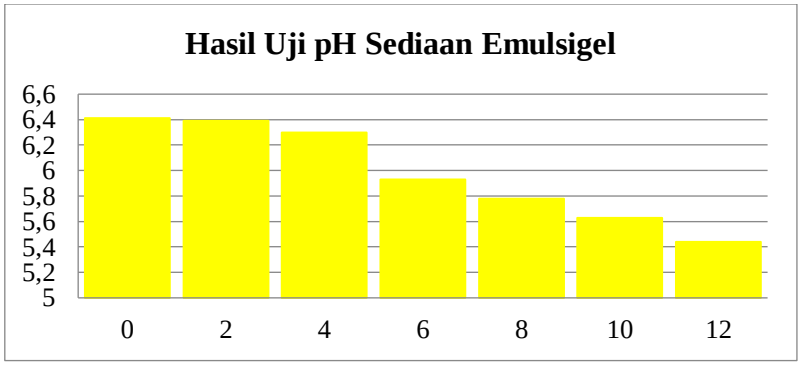

Figure 1. Results of pH Test of Emulsigel Preparations during 12 Weeks of Storage

The pH test aimed to ensure the safety of the preparation when used. Based on Figure 1., it can be seen that there is a decrease in pH during storage, but the pH value remains stable in the range of 4.5–6.5, which is in accordance with the pH of the skin and is safe to use.(Tranggono & Latifah, 2007).A drop in pH that is too far from the physiological pH can cause irritation or dry skin.(Young, 2002). Carbopol has an acidic solution pH, and the addition of TEA neutralizes its acidic nature. The decrease in pH during storage is likely due to the inability of TEA to completely neutralize carbopol.(Slamet et al., 2020).

- **Viscosity Test Results**

Viscosity measurements of the preparation were carried out after manufacturing and for 12 weeks of storage using a Brookfield viscometer. The results of the viscosity tests are shown in Figure 2.

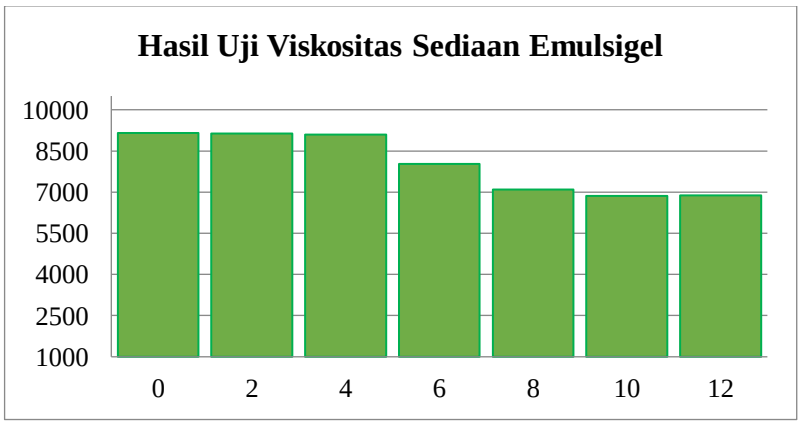

Figure 2. Viscosity Test Results of Emulsigel Preparations During 12 Weeks of Storage

Based on the results of the viscosity test in Figure 2., it can be concluded that as the storage time increased, the viscosity decreased. This decrease is caused by syneresis, where water molecules trapped in the matrix emerge, causing the liquid to move to the surface and resulting in a decrease in viscosity.(Astuti et al., 2017).

- **Emulsion Type Test Results**

Emulsion-type testing was performed to determine the type of emulsion used in the preparation. This method uses methylene blue, which is added to the preparation of transparent glass and then observed visually.(Directorate General of the POM, 1985). If methylene blue is evenly distributed, the preparation is oil-in-water (o/w). Conversely, if it is not evenly distributed, the preparation is water-in-oil (w/o) type. The results of the emulsion-type tests are shown in Figure 3.

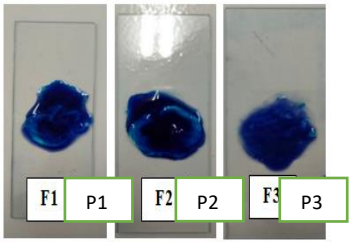

Figure 3. Emulsion Type Test Results

The results of the emulsion-type test showed that the methylene blue was evenly distributed. Thus, it can be concluded that clove leaf oil gel emulsion is an oil-in-water (o/a) emulsion. The test was carried out with three repetitions (triple).

- **Particle Size Test Results**

The particle size of the gel emulsion was determined using a Fritsch Particle Size Analyzer. Particle sizes of the gel emulsions were tested at weeks 0, 6, and 12. Based on the results of the particle size test of the clove leaf oil gel emulsion preparation, it was 8.3 µm at the beginning of manufacture, and there was an increase in particle size during 12 weeks of storage. The results of the particle measurement tests are listed in Table 3.

Table 3. Particle Size Test Results

Storage (Sunday)	Particle Size Gel Emulsion (µm)	Gel Emulsion Particle Size Distribution (nm)
0	8.3	2,162 - 20,801
6	11.73	1,066 - 23,962
12	14.01	0.605 - 23.962

- **Stability Test Results**

As shown in Figure 4., the clove leaf oil emulsion gel preparation was stable for 12 weeks of storage at room, low, and high temperatures. Color, odor, and shape did not change from the beginning of the observation period until 12 weeks of storage. Phase separation was also not observed, indicating that the clove leaf oil emulsion gel was stable for 12 weeks of storage.

Figure 4. Stability Test Results

- **Skin Irritation Test Results**

The results of the skin irritation test were carried out with the aim of determining whether the formulated preparation was safe and did not cause irritation to the skin. This test has met The Health Research Implementation Ethics Committee approved this study. Based on the results of the skin irritation test (Table 3.2), none of the panelists experienced irritation reactions. Therefore, it can be concluded that the emulsigel preparation is safe to use(Tranggono & Latifah, 2007).

Table 4. Skin Irritation Test Results

Panelists	Redness	Itchy	Roughening of the skin
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-

Information:

(−) : No irritation reaction

- **Antibacterial Activity Test Results of Clove Leaf Oil Emulsigel Preparation**

The antibacterial activity of the clove leaf oil emulsion gel was tested. Data from the measurement of the diameter of the inhibition zone of Staphylococcus epidermidis and Propionibacterium acnes bacteria against clove leaf oil emulsion gel are shown in Table 5., Figure 5., and Figure 6..

Table 5. Antibacterial Activity Test Results

Formula	Inhibition Zone Diameter* (mm)	
	Staphylococcus Epidermidis	***Propionibacterium acnes***
Emulsigel	18.03±0.11	17.33±0.25
Gel Base	00.00±0.00	00.00±0.00

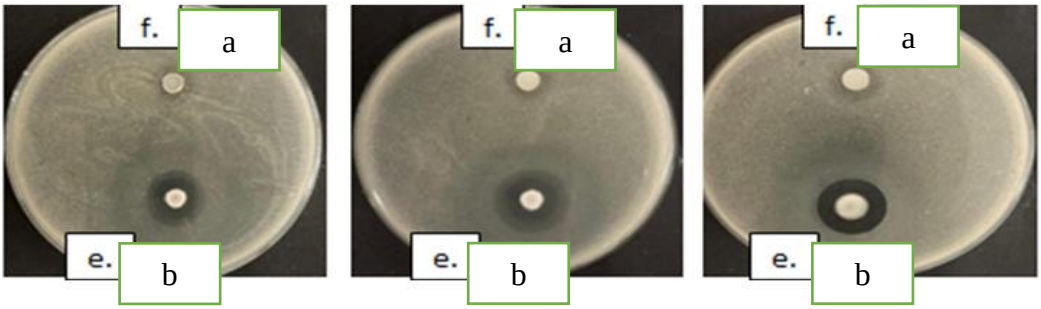

Figure 5. Diameter of inhibition zone of gel emulsion preparation (a) gel base (b) gel emulsion against Staphylococcus Epidermidis bacteria.

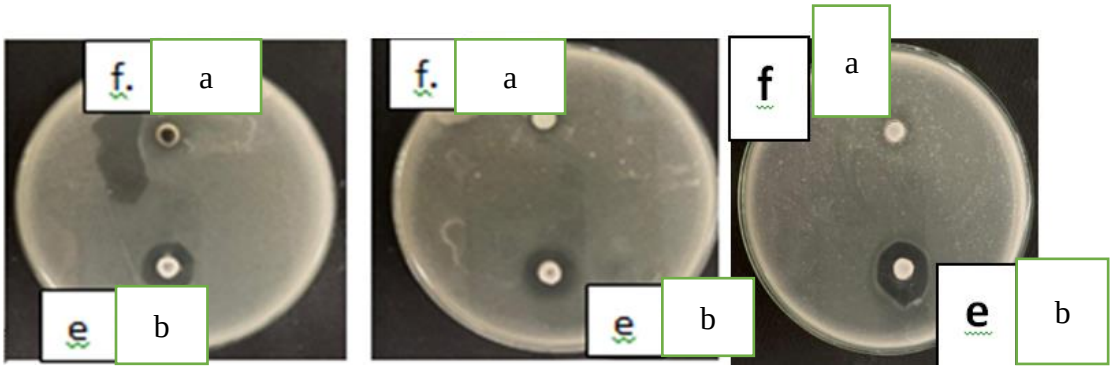

Figure 6. Diameter of inhibition zone of gel emulsion preparation (a) gel base (b) gel emulsion against Propionibacterium acnes bacteria.

Based on the results of the antibacterial activity test, it can be concluded that the gel base does not have an inhibition zone because it does not contain antibacterial properties against Propionibacterium acnes and Staphylococcus epidermidis, while the emulsion gel preparation had an inhibition zone diameter that was included in the very active inhibition category.

4. CONCLUSION

Based on the results of the research that has been conducted, it can be concluded that clove leaf oil with a concentration of 6% shows stability during storage for 12 weeks at various temperatures, namely low temperature (4°C), room temperature (25°C), and high temperature (40°C). In addition, clove leaf oil gel emulsion preparation has also been shown to have effective antibacterial activity against Propionibacterium acnes and Staphylococcus epidermidis, which are the main bacteria that cause skin problems such as acne. These results indicate that clove leaf oil-based gel emulsion is not only physically stable, but also has great potential to be used as a topical preparation for treating acne-prone skin problems.

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