

## Anti-Acne Activity of Jamblang Seed (*Syzygium cumini* L. Skeels) Ethanol Extract in Emulgel Preparation

### Aktivitas Anti Jerawat Ekstrak Etanol Biji Jamblang (*Syzygium cumini* L. Skeels) dalam Sediaan Emulgel

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#### Abstract

Jamblang (*Syzygium cumini* L. Skeels) contains antibacterial compounds and can be formulated into an emulgel form. This study aimed to determine the optimal concentration of ethanolic extract from jamblang seeds, develop emulgel formulations, and evaluate their antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The extraction was performed using 96% ethanol through maceration, followed by phytochemical screening and antibacterial testing using the well diffusion method. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, tannins, and triterpenoids. Antibacterial testing of jamblang seed extract against *S. aureus* and *S. epidermidis* at various concentrations demonstrated significant inhibition. The emulgel formulations were prepared using jamblang seed extract at concentrations of 5%, 10%, and 15%, with 3% HPMC as the gelling agent. The emulgel exhibited strong antibacterial activity, with the largest inhibition zones observed in the 15% formulation (F3), measuring  $21.44 \pm 0.21$  mm against *S. aureus* and  $19.02 \pm 0.60$  mm against *S. epidermidis*. Stability tests showed that the organoleptic properties and homogeneity remained stable. The pH, viscosity, adhesion, and spreadability tests met the criteria for quality preparations, and irritation tests showed no adverse skin reactions. Analysis of variance (ANOVA), followed by Tukey's post-hoc test, indicated significant differences ( $p < 0.05$ ) in antibacterial activity among all emulgel formulations. In conclusion, Formula F3 (15% extract) showed the best antibacterial activity and met all physical evaluation parameters, confirming that the jamblang seed emulgel is a high-quality and safe formulation. A paired t-test showed all parameters were statistically significant ( $p > 0.05$ ).

**Keywords:** Anti-acne, Jamblang seed, Emulgel

#### Abstrak

Jamblang (*Syzygium cumini* L. Skeels) mengandung senyawa antibakteri dan dapat diformulasikan dalam bentuk emulgel. Penelitian ini bertujuan untuk menentukan konsentrasi optimal ekstrak etanol dari biji jamblang, mengembangkan formulasi emulgel, serta mengevaluasi aktivitas antibakterinya terhadap *Staphylococcus aureus* dan *Staphylococcus epidermidis*. Ekstraksi dilakukan menggunakan etanol 96% melalui metode maserasi, dilanjutkan dengan penapisan fitokimia dan pengujian antibakteri menggunakan metode difusi sumuran. Penapisan fitokimia menunjukkan adanya kandungan alkaloid, flavonoid, saponin, tanin, dan triterpenoid. Formulasi emulgel dikembangkan menggunakan ekstrak biji jamblang dengan konsentrasi 5%, 10%, dan 15%, dengan penyesuaian HPMC sebesar 3% sebagai *agen gelling*. Emulgel menunjukkan aktivitas antibakteri yang kuat, dengan zona hambat terbesar pada formulasi emulgel (F3) 15% yang menghasilkan  $21,44 \pm 0,21$  mm terhadap *S. aureus* dan  $19,02 \pm 0,6$  mm terhadap *S. epidermidis*. Uji stabilitas menunjukkan bahwa sifat organoleptik dan homogenitas sediaan tetap stabil. Pengujian pH, viskositas, daya lekat, dan daya sebar memenuhi kriteria sediaan bermutu, serta uji iritasi menunjukkan tidak adanya reaksi merugikan pada kulit. Hasil uji ANOVA dilanjutkan dengan post hoc Tukey menunjukkan perbedaan yang signifikan ( $p < 0.05$ ) pada aktivitas antibakteri antara semua formula emulgel. Kesimpulannya, Formula F3 (ekstrak 15%) menunjukkan aktivitas antibakteri terbaik dan memenuhi semua parameter evaluasi fisik, sehingga memastikan bahwa emulgel biji jamblang merupakan formulasi yang bermutu dan aman. Hasil uji t-test berpasangan menunjukkan bahwa semua parameter berbeda secara signifikan ( $p > 0.05$ ).

**Kata Kunci:** Anti jerawat, Biji Jamblang, emulgel.



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## Introduction

The skin is the largest organ in the human body, located on the outer surface and visible under both normal and diseased conditions [1]. It functions as a protective barrier against external disturbances [2]. The skin contains normal flora, which consists of two types: transient flora and resident flora. Transient flora has lower pathogenicity and is less abundant than resident flora. When there is a disruption in the balance, both transient and resident flora can cause disease. Pathogenic bacteria from transient microorganisms found on the skin include *Salmonella* spp., *Shigella* spp., and *Escherichia coli*. Transient flora commonly found on the skin include *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes* [3].

Maintaining skin health is essential to prevent conditions such as tinea versicolor, boils, and acne. Acne is an abnormal skin condition caused by bacteria and inflammation, often accompanied by clogged pores, resulting in bulges due to excess oil production by the sebaceous glands [4]. The prevalence of acne is highest during puberty, and it frequently appears on the shoulders, chest, neck, back, and face. Due to its appearance, acne can be bothersome and may cause feelings of self-consciousness. The bacteria that can cause acne-related inflammation include *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The proportion of acne-causing bacteria is as follows: *Staphylococcus aureus* (39%), *Propionibacterium acnes* (33%), and *Staphylococcus epidermidis* (21%) [5].

Acne can be treated with antibiotics such as clindamycin, tetracycline, and erythromycin. However, the use of chemical-based drugs can cause side effects, such as skin irritation and antibiotic resistance, especially if not used at the correct dosage or according to proper guidelines. In addition to chemical-based treatments, acne can also be managed using topical preparations made from natural ingredients. These natural-based preparations are believed to cause fewer side effects [6]. One natural plant with antibacterial activity is the jamblang plant (*Syzygium cumini* L.). In general, research on jamblang has focused on the leaves and fruit, while the seeds remain underutilised. Jamblang seeds contain compounds such as alkaloids, flavonoids, saponins, tannins, triterpenoids, and amino acids, all of which have antibacterial properties.

Phytochemical analysis of jamblang seeds has identified compounds including gallic acid, alpha-phytosterols, tannins, and phytomeliad glucoside-5 [7]. The secondary metabolites found in jamblang seeds, particularly flavonoids and tannins, play a crucial role in inhibiting pathogenic bacteria [8]. Flavonoids exert their antibacterial activity by disrupting the bacterial cell wall matrix, increasing membrane permeability, and inhibiting vital protein synthesis [9]. Concurrently, tannins act as astringents that can denature bacterial cell proteins and inactivate essential enzymes, leading to cell lysis in both *Staphylococcus aureus* and *Staphylococcus epidermidis* [10]. While previous studies have primarily investigated the crude extract of jamblang leaves or fruits, the application of jamblang seed extract into advanced topical delivery systems remains highly limited.

## METHOD

### Materials and tools

The materials and equipment used in this study included: glassware, analytical balance, hot plate, laminar airflow cabinet (LAFB), rotary evaporator, viscometer, and aluminium foil. The chemicals and reagents consisted of: *Staphylococcus aureus*, *Staphylococcus epidermidis*, Nutrient Agar (NA) media, Mueller-Hinton Agar (MHA) media, 2N hydrochloric acid (HCl), concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), 10% ferric chloride (FeCl<sub>3</sub>), chloroform (CHCl<sub>3</sub>), 96% ethanol, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 0.9% sodium chloride (NaCl), and distilled water (aquadest). Other ingredients used for formulation included:

HPMC (Hydroxypropyl methylcellulose), liquid paraffin, Span 80, Tween 80, propyl paraben, methyl paraben, and propylene glycol.

### Sample collection

Jamblang seed samples were obtained from Lamreh Village, Masjid Raya District, Aceh Besar. Plant identification was carried out at the Bogoriense Herbarium, Indonesian Institute of Sciences (LIPI), Bogor Biology Research Centre [8]. The jamblang fruit is shown in Figure 1.



**Figure 1.** Jamblang seeds

### Preparation of simplicia

After the jamblang fruits were harvested, the seeds were extracted by separating them from the fruit flesh. The seeds were then washed with running water, drained, and thinly sliced. After being aerated, the seeds were dried in an oven at 50°C for 6 hours [9]. A total of 400 grams of simplicia powder was macerated with 3 litres of 96% ethanol as the solvent in a container. The container was covered with aluminium foil and stored in a location shielded from direct sunlight for 5×24 hours, with occasional stirring. After 5 days, the mixture was filtered using filter paper. The resulting macerate was stored, and the residue was subjected to remaceration with the remaining 1 litre of ethanol solvent for an additional 2 days. The macerate was then concentrated using a rotary evaporator at 50°C and 180 rpm [10].

### Phytochemical Profiling of Plant Extracts: Qualitative Analytical Approaches

Qualitative phytochemical screening of plant extracts is a foundational step in preliminary characterisation of bioactive constituents, employing standardised colourimetric and precipitation assays to detect major secondary metabolite classes. In the alkaloid detection protocol, 0.5 g of the extract was basified with ammonia solution to liberate free alkaloid bases, followed by acidification with 2N HCl and dilution with distilled water; the mixture was then heated for 2 minutes, cooled, and filtered to obtain a clear test solution.

The subsequent identification of alkaloids was achieved through parallel spot tests using three distinct precipitating reagents: Mayer's reagent (potassium mercuric iodide), Bouchardat's reagent (iodine-potassium iodide), and Dragendorff's reagent (potassium bismuth iodide), where the formation of creamy white, reddish-brown, or orange precipitates, respectively, indicated positive results. For flavonoid detection, 0.5 g of extract was dissolved in methanol, allowed to stand for 10 minutes, and filtered; the filtrate was then diluted with distilled water and partitioned with petroleum ether to remove chlorophyll and lipophilic interferences, after which the methanol layer was concentrated and reconstituted in ethyl acetate. An aliquot of this fraction was then subjected to the Shinoda test, wherein the addition of magnesium powder and concentrated hydrochloric acid to the ethanolic solution produced a characteristic pink, red, or orange colouration, confirming the presence of flavonoid aglycones.

The saponin assay involved the dissolution of 0.5 g of extract in hot distilled water, followed by cooling and vigorous shaking for less than 10 minutes, where the persistence of a stable foam layer even after the addition of 2N HCl indicated the presence of saponin glycosides, reflecting their surface-active properties and resistance to acid hydrolysis. Tannin content was evaluated by macerating 0.5 g of extract in distilled water for 15 minutes, filtering, and diluting the filtrate until nearly colourless, followed by the addition of 2 drops of 10% ferric chloride (FeCl<sub>3</sub>), with the development of a blue-black, green, or brownish precipitate confirming the presence of hydrolyzable or condensed tannins.

Lastly, the triterpenoid/steroid screening was performed by treating 0.5 g of extract with Liebermann-Burchard reagent (acetic anhydride and concentrated sulfuric acid), where the appearance of a pink-yellow colouration was interpreted as a positive indication for triterpenoids. In contrast, the formation of a green or blue-green colour denoted the presence of steroidal compounds, based on the differential colourimetric

responses arising from the sterol nucleus and its side-chain modifications. Collectively, these qualitative assays provide a rapid, cost-effective, and reproducible preliminary assessment of phytochemical diversity, guiding subsequent fractionation, bioassay-guided isolation, and chemotaxonomic profiling of medicinal plant extracts.

### Jamblang seed ethanol extract emulgel formulation

The emulgel formulations were prepared using jamblang seed extract at concentrations of 5%, 10%, and 15%, which were selected based on the effective antibacterial efficacy shown in the preliminary screening. The detailed composition of each formula is presented in Table 1.

**Table 1.** Formulation Emulgel

| No | Ingredients                     | Concentrations (%) |        |        |        |
|----|---------------------------------|--------------------|--------|--------|--------|
|    |                                 | F0                 | F1     | F2     | F3     |
| 1  | Jamblang seed ethanolic extract | 0                  | 5      | 10     | 15     |
| 2  | HPMC                            | 3                  | 3      | 3      | 3      |
| 3  | Liquid Paraffin                 | 5                  | 5      | 5      | 5      |
| 4  | Span 80                         | 0,42               | 0,42   | 0,42   | 0,42   |
| 5  | Tween 80                        | 1,08               | 1,08   | 1,08   | 1,08   |
| 6  | paraben                         | 0,02               | 0,02   | 0,02   | 0,02   |
| 7  | Methyl paraben                  | 0,18               | 0,18   | 0,18   | 0,18   |
| 8  | Propylene glikol                | 10                 | 10     | 10     | 10     |
| 9  | Aquadest                        | Ad 100             | Ad 100 | Ad 100 | Ad 100 |

All the materials needed are weighed beforehand. The gel mass was made by dispersing the HPMC gradually in hot water at 80°C, allowing it to stand for 20-30 minutes until the HPMC swelled, then crushing it to form a gel base. Methyl paraben and paraben profile were dissolved in propylene glycol, then mixed with the gel base. Jamblang seed ethanol extract was added.

All the materials required were weighed in advance. The gel base was prepared by gradually dispersing the HPMC in hot water at a temperature of 80°C and allowed to stand for 20-30 minutes until the HPMC swelled. The mixture was then triturated to form a gel base. Methyl paraben and propyl paraben were dissolved in propylene glycol and then incorporated into the gel base. The ethanol extract of jamblang seeds was added gradually while stirring until a homogeneous mixture was obtained (mixture 1). The emulsion base was prepared by separately heating the oil phase (Span 80 and liquid paraffin) and the water phase (Tween 80) at 70°C. The two phases were then combined in a mortar containing mixture 1. The mixture was blended for approximately 45 minutes until homogeneous, resulting in the formation of an emulgel base. The gel preparation was then transferred into a container and evaluated [11].

### Comprehensive Physicochemical and Stability Evaluation of Formulated Emulgel

The quality assurance of the developed emulgel formulation was systematically conducted through a series of standardised physicochemical tests, encompassing organoleptic, homogeneity, pH, dispersion, viscosity, adhesion, stability, irritation, and emulsion type assessments, all of which are critical parameters for ensuring product performance, patient compliance, and shelf-life integrity.

The organoleptic evaluation was performed by visual and tactile observation of the formulated emulgel, with particular attention paid to its colour, shape, and texture, providing an initial qualitative profile of the preparation's aesthetic acceptability [12]. Homogeneity testing was performed by spreading 0.5 g of the emulgel onto a glass slide, covering it with a coverslip, and subsequently examining the preparation for coarse particles or agglomerates, thereby ensuring uniform distribution of the dispersed phase throughout the vehicle [12].

The pH measurement was conducted using a calibrated pH meter, wherein 1 g of the sample was dissolved in 100 mL of distilled water, equilibrated for 2 hours, and the resulting pH value was recorded to ascertain the dermatological compatibility of the formulation with the skin's physiological pH range [12]. Dispersion characteristics were evaluated using a parallel glass plate method, where 0.5 g of the preparation was placed between two circular glass plates, subjected to incremental loading up to a total weight of 200 g, and after 1 minute, the diameter of the spread area was measured using a centimetre-scaled ruler, indicating the emulgel's ease of application and spreadability [13].

The viscosity profile was determined using a Brookfield cone viscometer, employing an appropriate spindle number and rotational speed to ensure the sample was fully submerged, thereby yielding reproducible rheological data essential for predicting the formulation's flow behaviour and extrusion properties [14]. Adhesion testing was performed by placing 0.5 g of the emulgel onto the smooth surface of a glass slide within a predetermined area, overlaying it with a second glass slide, applying a 150 g load for 1 minute, and subsequently measuring the detachment time after the load was removed, which quantifies the bioadhesive strength of the preparation—a crucial parameter for topical drug retention [15].

The accelerated stability study was executed using the freeze-thaw cycling method, wherein the emulgel was alternately exposed to  $45\pm 2^\circ\text{C}$  for 24 hours and  $-5\pm 2^\circ\text{C}$  for 24 hours per cycle, repeated over 6 cycles, followed by reassessment of organoleptic properties, homogeneity, and pH to detect any physical or chemical instability induced by thermal stress [16]. The dermal irritation potential was evaluated through an open patch test applied to a  $2.5 \times 2.5$  cm area of the forearm for 2 days, involving 30 healthy female volunteers aged 20–30 years who met the inclusion criteria of no history of allergies and voluntary participation, while excluding pregnant individuals, those using immunomodulators or corticosteroids, participants in concurrent similar studies, and those with localized forearm skin conditions such as tattoos, scars, sunburn, or dermatoses; post-application visual observations were scored as erythema/redness (+), pruritus/itching (++), or edema/swelling (+++) for positive reactions, whereas the absence of any adverse response was classified as non-irritating (-) [4].

Lastly, the emulsion type was identified through a dilution method: oil-in-water (O/W) emulsions exhibited miscibility with water, and water-in-oil (W/O) emulsions with oil, with subsequent microscopic examination confirming the continuous and dispersed phases, thereby validating the structural integrity of the emulsified system [10]. Collectively, these comprehensive quality control parameters ensure that the formulated emulgel meets the requisite standards for topical pharmaceutical products in terms of physical stability, rheological behaviour, skin safety, and application efficacy.

### Activity test of antibacterial emulgel preparations

The tested microbes were *S. aureus* and *S. epidermidis* bacteria. 1 mL of bacterial suspension was added to each Petri dish and mixed with 30 mL of MHA media. The petri dish was then rotated in a circular motion following a figure-eight pattern to ensure thorough mixing of the media and suspension, and allowed to solidify. A well with a diameter of 6 mm was then created using a cork borer. Each well was filled with 30  $\mu\text{L}$  of emulgel preparation at concentrations of 5%, 10%, and 15%. The media containing the bacteria were incubated at  $37^\circ\text{C}$  for 24 hours. The clear zone formed was observed, and the diameter was measured using a calliper (in mm) [17].

## Results and Discussion

### Phytochemical screening

Phytochemical test of jamblang seed extract was carried out to determine the secondary metabolite content of jamblang seed extract. Phytochemical tests were carried out qualitatively, including alkaloid, flavonoid, saponin, tannin, and steroid/triterpenoid tests. Phytochemical test results are shown in Table 2.

**Table 2.** Qualitative Phytochemical Profile of Jamblang (*Syzygium cumini*) Seed Extract

| No. | Phytochemical test | Result                                                | Description |
|-----|--------------------|-------------------------------------------------------|-------------|
| 1   | Flavonoid          | Formation of orange colouration                       | +           |
| 2   | Alkaloid           | - Mayer's reagent: White precipitate                  | +           |
|     |                    | - Bouchardat's reagent: Brown precipitate             | +           |
|     |                    | - Dragendorff's reagent: Yellowish-orange precipitate | +           |
| 3   | Tanin              | Formation of blackish-blue colouration                | +           |
| 4   | Saponin            | Persistent foam formation                             | +           |
| 5   | Triterpenoid       | Formation of yellowish-red colouration                | +           |

Note: +: Contains secondary metabolites

### Physical evaluation of preparations

The jamblang seed extract emulgel formulation is a modified version of the research conducted by Daud and Suyanti [18]. The modification involved changing the active ingredient from patchouli oil to the ethanol extract of jamblang seeds, and reducing the HPMC concentration from 6% to 3%. The preparation using a 6%

concentration produced an emulgel with a hard and inhomogeneous texture, making it difficult to apply to the skin. Therefore, the formulation was modified by lowering the HPMC concentration to 3% to produce a better preparation that was homogeneous and easy to apply to the skin. The concentration of jamblang seed extract used in the preparation was 0%, 5%, 10%, and 15%. The results of the organoleptic test on the Emulgel F0 preparation showed that it was white in colour, in the form of an emulgel, and had a smooth texture. F1 had a cream colour, an emulgel form, a distinctive aroma of jamblang seeds, and a smooth texture. F2 was brownish cream in colour, had an emulgel form, a distinctive aroma of jamblang seeds, and a smooth texture. F3 was brown, in emulgel form, had a distinctive aroma of jamblang seeds, and a smooth texture.

Homogeneity was assessed to determine whether the emulgel preparation was homogeneous and whether its components were evenly mixed. The homogeneity of emulgel preparations F0, F1, F2, and F3 was assessed and showed homogeneous results, with no coarse grains observed when the preparations were smeared on the slide.

The pH test results indicated that the preparations F0, F1, F2, and F3 met the normal skin requirements of 4.5–6.5[11], suggesting that they did not cause dry or irritated skin, even with different concentrations of jamblang seed extract. Therefore, the jamblang seed extract emulgel preparation is considered safe for use. The viscosity test aimed to determine the viscosity of the preparation. A good topical preparation should not be too runny or too thick [14]. The results of the viscosity test showed that variations in the concentration of jamblang seed extract affected the viscosity. The higher the concentration of jamblang seed extract added, the lower the viscosity value. Viscosity refers to resistance to flow; the thicker the liquid, the greater the force required for the liquid to flow [14]. The results of the viscosity test for F0, F1, F2, and F3 preparations were 2396, 2381, 2373, and 2369 cPs, respectively.

The results of the spreadability test for all jamblang seed extract emulgel preparations met the required standards. Preparations F0 and F1 were classified as semistiff, with a diameter of less than 5 cm, while F2 and F3 were classified as semifluid, as their diameters ranged from 5 cm to 7 cm. This is directly proportional to the viscosity values observed; the lower the viscosity value, the higher the dispersion value [15]. The adhesion test was carried out to determine the ability of the preparation to adhere to the skin. For semi-solid preparations, good adhesion should last for more than 1 second [19]. Based on statistical analysis using the paired *t*-test, the results for pH, viscosity, adhesion, and spreadability showed  $p > 0.05$ . The *t*-test for the pH parameter showed a *p*-value before testing of (0.256) and a *p*-value after testing of (0.297), both  $> 0.05$ . The *t*-test for the viscosity parameter showed a *p*-value before testing of (0.214) and a *p*-value after testing of (0.321), both  $> 0.05$ . The adhesion test for the adhesion parameter showed a *p*-value before testing of (0.272) and a *p*-value after testing of (0.303), both  $> 0.05$ . The *t*-test for the spreadability parameter showed a *p*-value before testing of (0.282) and a *p*-value after testing of (0.292), both  $> 0.05$ .

The emulsion type test was conducted to determine the type of emulsion in the jamblang seed extract emulgel preparation. The type of emulsion produced by F1, F2, and F3 was oil-in-water (O/W). An O/W emulsion consists of oil droplets dispersed in water, with oil serving as the internal phase and water as the external phase. The results of the O/W emulsion type showed that this emulsion has the advantage of being easy to wash [10].

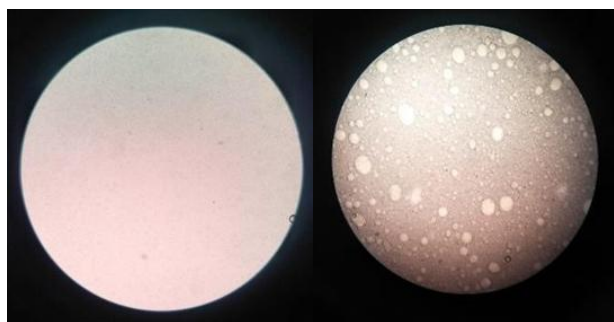


**Figure 2.** Emulgel preparations

The stability test was conducted for 6 cycles (12 days). Each cycle consisted of 24 hours at 45°C followed by 24 hours at -5°C, and the preparation was observed for any changes in organoleptic properties and homogeneity. After completing the cycling test, the results showed that the preparation remained stable, with no observable changes. From the average results of the tests for pH, viscosity, adhesion, and dispersibility, the values remained within the required range, although a slight decrease was observed [16].

The irritation test was performed using the open patch test method on the inside of the forearm of 30 panellists. The results of the irritation test for the emulgel preparations F0, F1, F2, and F3 indicated that none

of the panellists experienced irritation, such as itching, redness, or swelling, on the treated skin [20]. These findings suggest that the emulgel preparation is safe for use on the skin, as shown in Figure and Table 2.



**Figure 3.** Microscopic observation of emulsion type.

**Table 3.** Evaluation of Emulgel Before and After Stability Test

| Parameters         | Condition | F0          | F1                         | F2                              | F3                              |
|--------------------|-----------|-------------|----------------------------|---------------------------------|---------------------------------|
| Colour             | Before    | White       | Cream                      | Brownish cream                  | Brown                           |
|                    | After     | White       | Cream                      | Brownish cream                  | Brown                           |
| Form               | Before    | Emulgel     | Emulgel                    | Emulgel                         | Emulgel                         |
|                    | After     | Emulgel     | Emulgel                    | Emulgel                         | Emulgel                         |
| Aroma              | Before    | Base        | Distinctive jamblang scent | Distinctive jamblang seed scent | Distinctive jamblang seed scent |
|                    | After     | Base        | Distinctive jamblang scent | Distinctive jamblang seed scent | Distinctive jamblang seed scent |
| Texture            | Before    | Smooth      | Smooth                     | Smooth                          | Smooth                          |
|                    | After     | Smooth      | Smooth                     | Smooth                          | Smooth                          |
| Homogeneity        | Before    | Homogeneous | Homogeneous                | Homogeneous                     | Homogeneous                     |
|                    | After     | Homogeneous | Homogeneous                | Homogeneous                     | Homogeneous                     |
| pH                 | Before    | 5,79 ± 0,09 | 5,59 ± 0,06                | 5,45 ± 0,04                     | 5,43 ± 0,08                     |
|                    | After     | 6,6 ± 0,07  | 6,55 ± 0,04                | 6,46 ± 0,02                     | 6,13 ± 0,05                     |
| Viscosity (cPs)    | Before    | 2396 ± 0,1  | 2381 ± 0,01                | 2373 ± 0,01                     | 2369 ± 0,01                     |
|                    | After     | 1786 ± 0,01 | 1743 ± 0,01                | 1738 ± 0,01                     | 1724 ± 0,01                     |
| Adhesion (seconds) | Before    | 3,83 ± 0,1  | 3,74 ± 0,05                | 3,06 ± 0,53                     | 2,65 ± 0,11                     |
|                    | After     | 3,40 ± 0,1  | 3,38 ± 0,1                 | 1,86 ± 0,1                      | 2,01 ± 0,19                     |
| Spreadability (cm) | Before    | 4,7 ± 0,2   | 4,64 ± 0,1                 | 5,42 ± 0,1                      | 5,78 ± 0,1                      |
|                    | After     | 4,92 ± 0,3  | 4,88 ± 0,3                 | 6,08 ± 0,2                      | 6,62 ± 0,1                      |
| Emulsion type test | Before    | O/W         | O/W                        | O/W                             | O/W                             |
|                    | After     | O/W         | O/W                        | O/W                             | O/W                             |

Note. All parameters are statistically significant,  $p > 0.05$

### Antibacterial activity test

The antibacterial activity test of the preparation on *S. aureus* and *S. Epidermidis* bacteria at various concentrations of jamblang seed extract was 0, 5, 10 and 15%. The results of the antibacterial activity of the emulgel preparation can be seen in Table 4.

**Table 4.** The results of the antibacterial activity of jamblang seed extract emulgel preparations

| Concentration (%) | Average Inhibition zones (mm) ± SD |                                   |
|-------------------|------------------------------------|-----------------------------------|
|                   | <i>Staphylococcus aureus</i>       | <i>Staphylococcus epidermidis</i> |
| 0                 | 0,00 ± 0,00 <sup>a</sup>           | 0,00 ± 0,00 <sup>a</sup>          |
| 5                 | 14,16 ± 0,10 <sup>a</sup>          | 14,79 ± 0,06 <sup>a</sup>         |
| 10                | 20,52 ± 0,12 <sup>b</sup>          | 16,09 ± 0,5 <sup>a</sup>          |
| 15                | 21,44 ± 0,21 <sup>c</sup>          | 19,02 ± 0,6 <sup>b</sup>          |
| Positive Control  | 21,59 ± 0,09 <sup>c</sup>          | 34,33 ± 1,5 <sup>c</sup>          |
| Negative Control  | 0.00 ± 0.00 <sup>a</sup>           | 0.0 ± 0.00 <sup>a</sup>           |

Note: (n=3), ( $p < 0.05$ )

The activity test of the emulgel preparation used clindamycin as a positive control. The results showed that the diameter of the inhibition zone for *S. aureus* was 21.59 mm ± 0.09, and for *S. epidermidis*, it was 34.33 mm ± 1.5. The largest inhibition zone diameter was observed at a concentration of 15%, with values of 21.44 ± 0,21 mm for *S. aureus* and 19.02 ± 0,6 mm for *S. epidermidis*. The smallest inhibition zone diameter was observed

at a concentration of 5%, with values of  $14.16 \pm 0,10$  mm for *S. aureus* and  $14.79 \pm 0,06$  mm for *S. epidermidis*. The antibacterial activity test of the extracts demonstrated an inhibitory zone against *S. aureus* and *S. epidermidis*, with the largest inhibition zone diameter at a concentration of 15%, measuring  $27.46 \pm 0,49$  mm for *S. aureus* and  $19.76 \pm 0,93$  mm for *S. epidermidis*. The ANOVA results showed a significant difference ( $p < 0.05$ ) in antibacterial activity among all emulgel formulations. Tukey's post-hoc test revealed that the F3 formulation (15%) differed significantly from F1 (5%) and F2 (10%) but did not differ significantly from the positive control for the *S. aureus* bacteria ( $p > 0.05$ ). In contrast, the F3 formulation differed significantly from the positive control against *S. epidermidis* ( $p < 0.05$ ).

The emulgel formulations exhibit significant dose-dependent antibacterial activity against both *Staphylococcus aureus* and *Staphylococcus epidermidis*. Formula F3 (15% extract concentration) demonstrated the highest efficacy, achieving an antibacterial potency against *S. aureus* ( $p > 0.05$ ) compared to the positive control. The compounds detected by GC-MS analysis contribute to the extract's activity [8]. Results from the activity test showed a decrease in the inhibition zone diameter compared to the extract. This reduction was likely due to the influence of the ingredients in the preparation, such as HPMC and propylene glycol. HPMC can increase the viscosity of the preparation, and the higher the viscosity value, the greater the resistance to flow. Additionally, propylene glycol functions as a humectant that binds water molecules within the formulation, which can further intensify the density of the gel network. This resistance may block the release of the active substance, leading to a decrease in the diameter of the inhibition zone [21].

## Conclusion

The ethanol extract of jamblang seeds was successfully formulated into a stable emulgel that met the required physical quality standards. The emulgel containing 15% extract (F3) exhibited the strongest antibacterial activity against *S. aureus* and *S. epidermidis*, with inhibition zones of  $21.44 \pm 0,21$  mm and  $19.0 \pm 20,6$  mm, respectively. This study demonstrates the potential of jamblang seed emulgel as an alternative treatment for acne, although its antibacterial activity was lower than that of the pure extract.

## Conflict of Interest

Authors declare that there are no conflicts of interest.

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