Antibacterial activity of cream, ointment, and emulgel of *Ocimum basilicum* L. essential oil against *Propionibacterium acnes*

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**ABSTRACT**

*Propionibacterium acnes* is bacteria that is commonly found on healthy human skin. However, unbalanced hormonal conditions can lead to excessive growth of *P. acnes*, a bacteria causing acne. Previous research reported that *Ocimum basilicum* L. essential oil (BEO) has activity against *P. acnes*. Therefore, this study aims to make BEO into cream, ointment, and emulgel forms to determine its properties and antibacterial activity on those topical drug delivery systems. The preparations consisted of variations in BEO concentrations of 5%, 10%, and 15%. The three preparations were evaluated based on organoleptic, homogeneity, pH value, adhesion, and spreadability. The antibacterial activity test of the three preparations against *P. acnes* was carried out by the good diffusion method with the observation of the diameter of the inhibition zone (in mm). The produced cream, ointment, and cream had a typical BEO aroma, yellowish white color, and homogeneous. Based on organoleptic consistency, the texture of the thicker and longer adhesion was ointment > emulgel > cream, respectively. The pH value of the topical preparation was 5-6 and easily leveled. Antibacterial activity against *P. acnes* from the strongest was ointment > cream > emulgel, with a value of 14.30 ± 0.30 mm; 10.54 ± 0.01 mm; 3.90 ± 0.04 mm, respectively.

**Keywords:** Cream; emulgel; *Ocimum basilicum* L.; ointment; *Propionibacterium acnes*

**INTRODUCTION**

*Ocimum basilicum* L. (*O. basilicum* L.) is traditionally used as a spice, as well as in the medicinal and perfume industries. This perennial plant grows in tropical and subtropical regions, such as India, Afghanistan, Indonesia, Pakistan, Iran, Africa, and South America (El-Soud et al., 2017; Moghaddam et al., 2011). This herb from the Lamiaceae tribe has antibacterial activity both against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Propionibacterium acnes* and Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* (Hapsari & Feroniasanti, 2019; Moghaddam et al., 2011). Based on the results of Hapsari & Feroniasanti (2019), *O. basilicum* attrition oil has MIC and MBC values against *P. acnes* of 2% v/v and 3.5% v/v, respectively.

Bacteria that is commonly found on healthy human skin is *P. acnes*, a Gram-positive and rod-shaped form of bacteria (Borrel et al., 2019; O’Neill et al., 2020). In normal numbers, this bacteria, which has the synonym *Cutibacterium acnes*, along with *Corynebacteria* and *Staphylococci*, is protective of the skin (Platsidaki & Dessinioti, 2018). If there is a hormonal imbalance, sebum production will increase which is accompanied by hyperkeratinization of the follicular duct (Alkhawaja et al., 2020). The phenomenon causes the accumulation of dead skin and fatty acids, favoring anaerobic and lipophilic conditions in the skin (Castillo et al., 2019). Under anaerobic conditions, this excessive sebum will be hydrolyzed by *P. acnes* into propionic acid (Bojar & Holland, 2004). Furthermore, this organic acid can irritate the follicular wall and induce the release of inflammatory mediators (Movita, 2013). Therefore, the overgrowth of *P. acnes* can cause inflammation resulting in acne, eye infections, and abscesses in the human brain (Patel et al., 2009). Acne on the skin is common on the face, neck, chest, and back (Kuehnast et al., 2018).

Moreover, *P. acnes* produces lipase enzymes, chemotactic factors, metalloproteases, and porphyrins that will interact with oxygen molecules. These interactions produce reactive oxygen species (ROS) and free radicals which cause damage to the keratinocytes that make up the skin epidermis. The biofilm form of *P. acnes* stimulates the production of interleukin 1 (IL-1) in keratinocytes, triggering the formation of microcomedones and resistance to antimicrobial agents (Beylot et al., 2014).

The usage of antibiotics, such as erythromycin and clindamycin, is commonly used in the treatment of bacterial acne (Powale et al., 2022). However, the efficacy of this treatment...
Antibacterial activity of cream, ointment, and emulgel of Ocimum basilicum L.

METHODOLOGY
Preparation of plant material
O. basilicum herb was obtained from Bugel, Panjatan, Kulon Progo, Yogyakarta. A number of 2.5 kg of this herb (in triplicate) was extracted by water vapor distillation method for 5 hours in a distillation flask. The total sample used in the distillation was 7.5 kg. Anhydrous Na2SO4 was added to BEO to absorb water footprint content.

Formulation
The topical preparations to be made in this study include cream, ointment, and emulgel. The type of emulsion used to make the three preparations is oil in water. The composition of the ingredients can be seen in Table I.

The cream was made by heating the oil phase and water phase in separate containers. The oil phase consists of stearic acid, cera alba, and vaseline album (General Labora), while the water phase consists of triethanolamine, propylene glycol (Merck), methyl paraben (General Labora), and distilled water. The dissolved water phase was added to the oil phase in a warm mortar and mixed until homogeneous. The cooled cream base was added with BEO (Wiguna, 2016).

The ointment was prepared by melting PEG 400 and PEG 400 (Bratachem) at 70°C. After the mixture returned to room temperature, BEO was added to the mixture, hereafter called mixture A. In a different container, propyl paraben (General Labora) was suspended in distilled water until homogeneous (mixture B). Mixture A was added to mixture B gradually and stirred until homogeneous (Rakhim, 2016).

The emulgel was made by dividing the material into three parts, namely the oil phase, water phase, and gel base. The oil phase consisted of liquid paraffin and span 80 (Merck) was heated at 70°C and stirred until homogeneous, then let stand until it reached room temperature. At the next stage, BEO was added to the oil phase. The aqueous phase consisted of tween 80 (Bratachem), methyl paraben, propyl paraben, propylene glycol, and some distilled water. The mixing process was performed at 70°C. The gel base consisted of carbopol 940 (Bratachem), triethanolamine, and distilled water. Carbopol 940 was developed in a portion of distilled water and then triethanolamine was added which had been dissolved in a little distilled water. Stirring of the gel base was performed until the expected gel mass was formed. The homogeneous oil phase was added to the water phase gradually until a homogeneous emulsion was formed. The emulsion formed is

decreases when antibiotic resistance to *P. acnes* occurs and kills beneficial microbes (Bojar & Holland, 2004; O’Neill et al., 2020; Waranuch et al., 2019). Resistance to both antibiotics occurred in Spain (92.4%), Greece (75.3%), and Italy (59.5%) (Alkhwaja et al., 2020). As a result, greater antibacterial strength is needed to treat subsequent infections (Madelina & Sulistiyani, 2018). On October 04, 2022, in Indonesia, a number of 46 imported cosmetic products were withdrawn from circulation due to clindamycin content (BPOM RI, 2020, 2022).

One of the alternative natural products for infection treatment is BEO, which contains many secondary metabolites that synergize and complement each other in killing bacterial cells (Liu et al., 2019; Mera et al., 2019; Purwanto & Irianto, 2022). This oil contains neral compounds (41.45%), citral (33.29%), α-humulene (4.46%), β-caryophyllene (2.62%), linalool (2.45%) and germacrene-d (2.32%). The antibacterial effect of citral is through the mechanism of decreasing intracellular adenosine triphosphate (ATP) which plays an important role in bacterial cell respiration. Linalool, a terpene alcohol, causes damage to the bacterial cell membrane resulting in leakage of intracellular material and causing cell death. Compounds of α-humulene, β-caryophyllene, and germacrene-d belong to the sesquiterpene group and can disrupt the permeability of bacterial cell membranes so that bacterial growth is disrupted (Hapsari & Feronia, 2019; Purwanto & Irianto, 2022).

The usage of BEO on the skin as an anti-acne drug requires topical preparations that have quality, safety, and benefits. The parameters of the physical properties of the preparation are not enough to guarantee that the preparation can easily release active substances and cause effects. Therefore, in this study, we studied the preparation characteristics and antibacterial activity of cream, ointment, and emulgel of BEO. We preferred those three dosage forms because they are commonly used as a pharmaceutical dosage forms for topical infective diseases. The cream dosage form in this research was oil in a water type (o/w) to reduce its viscosity and get good spreadability (Amelia et al., 2020). Meanwhile, the ointment in this research was formulated as hydrophilic type and high viscosity to get better stickiness and easy to rinse. Whereas emulgel has properties as an emulsion (as cream) but has a higher viscosity (as ointment). This third dosage form has good spreadability and is easy to rinse.

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mixed into the gel base gradually while stirring with a stamper until homogeneous (Irianto et al., 2020).

Physical evaluation

The resulting preparation is then evaluated based on its physical properties including organoleptic, homogeneity, pH value, spreadability, and adhesiveness. The organoleptic test was carried out by observing the color, aroma, and consistency of the resulting preparation. This observation was carried out by all of the authors. The homogeneity test was carried out by observing organoleptically the presence/absence of granules in a preparation of 0.1 g which had been applied to a glass object. Observation of pH value was conducted with universal pH. A small part of each dosage form was applied to universal pH paper and then was compared to the universal standard. Observation of spreadability was carried out on the average diameter of the spread of 0.5 g of preparation that had been loaded up to 250 g, then the diameter was measured in cm. Observation of adhesion was carried out on the time required for the preparation (as much as 0.5 g) to attach two glass objects that had been loaded with 1 Kg for 5 minutes, and measured in seconds (Irianto et al., 2020).

Antibacterial activity

The bacterial culture used in this study was P. acnes ATCC 6919 in Brain Heart Infusion (Merck) (BHI) media. The preparation of bacterial suspension is equivalent to 1,5x10⁸ CFU/mL. Bacterial cultivation was carried out using the pour plate method, while the antibacterial test method was well diffusion. The negative control was the preparation base without BEO, while the positive control was Medi-Klin® gel containing clindamycin phosphate 1%. The samples were cream, ointment, and emulgel containing BEO. Incubation was carried out under anaerobic conditions, at 37°C for 12-16 hours, while observations were made of the clear zone around the wells expressed as the diameter of the inhibition zone (mm). The inhibition zone diameter (Ø) of each sample was then grouped into weak (Ø < 5 mm); medium (5 mm ≤ Ø < 10 mm); strong (10mm ≤ Ø < 20 mm);

### Table I. Ingredients in the preparation of O. basilicum cream, ointment and emulgel

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration (%)</th>
<th>Cream</th>
<th>Ointment</th>
<th>Emulgel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>BEO</td>
<td></td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Stearic acid</td>
<td></td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Cera alba</td>
<td></td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Vaseline album</td>
<td></td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td></td>
<td>1,5</td>
<td>1,5</td>
<td>1,5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td></td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td></td>
<td>0,3</td>
<td>0,3</td>
<td>0,3</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>Ad 100</td>
<td>Ad 100</td>
<td>Ad 100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration (%)</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEO</td>
<td></td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>PEG 4000</td>
<td></td>
<td>34</td>
<td>34</td>
<td>34</td>
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<tr>
<td>PEG 400</td>
<td></td>
<td>51</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td></td>
<td>0,08</td>
<td>0,08</td>
<td>0,08</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>Ad 100</td>
<td>Ad 100</td>
<td>Ad 100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration (%)</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEO</td>
<td></td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td></td>
<td>1,5</td>
<td>1,5</td>
<td>1,5</td>
</tr>
<tr>
<td>Span 80</td>
<td></td>
<td>1,4</td>
<td>1,4</td>
<td>1,4</td>
</tr>
<tr>
<td>Tween 80</td>
<td></td>
<td>3,6</td>
<td>3,6</td>
<td>3,6</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Methyl paraben</td>
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<td>0,18</td>
<td>0,18</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td></td>
<td>0,02</td>
<td>0,02</td>
<td>0,02</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>Ad 100</td>
<td>Ad 100</td>
<td>Ad 100</td>
</tr>
</tbody>
</table>
**Antibacterial activity of cream, ointment, and emulgel of Ocimum basilicum L.**

**Table II. Organoleptic characteristics and homogeneity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organoleptic</th>
<th>Homogeneity</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Aroma</td>
<td>Consistency</td>
</tr>
<tr>
<td>CREAM</td>
<td>F1</td>
<td>Yellowish white</td>
<td>Typical <em>O. basilicum</em></td>
</tr>
<tr>
<td>OINTMENT</td>
<td>F4</td>
<td>Yellowish white</td>
<td>Typical <em>O. basilicum</em></td>
</tr>
<tr>
<td></td>
<td>F5</td>
<td>Yellowish white</td>
<td>Typical <em>O. basilicum</em> (+)</td>
</tr>
<tr>
<td></td>
<td>F6</td>
<td>Yellow</td>
<td>Typical <em>O. basilicum</em> (++)</td>
</tr>
<tr>
<td>EMULGEL</td>
<td>F7</td>
<td>Pale yellow</td>
<td>Typical <em>O. basilicum</em></td>
</tr>
<tr>
<td></td>
<td>F8</td>
<td>Pale yellow</td>
<td>Typical <em>O. basilicum</em> (+)</td>
</tr>
<tr>
<td></td>
<td>F9</td>
<td>Yellow</td>
<td>Typical <em>O. basilicum</em> (++)</td>
</tr>
</tbody>
</table>

*(+) sign indicated the comparison of each parameter in the same dosage form. The higher amount of sign (+) means the higher value of the intended parameter.*

and very strong (Ø ≥ 20 mm) antibacterial categories (Purba et al., 2022).

**RESULT AND DISCUSSION**

**Characteristics of cream, ointment, and emulgel containing Ocimum basilicum essential oil**

The resulting cream, ointment, and emulgel have a distinctive aroma of *O. basilicum* because they contain the main volatile and aromatic compound, linalool. If inhaled, this chemical compound provides relaxation and sedative effects (Fattahi et al., 2019; Kim et al., 2022). The higher the concentration of BEO, the more pungent the aroma produced (Table II). The base of the cream, ointment, and emulgel was white, but after BEO was applied, the color became yellowish-white. This color arises from BEO which is yellowish (Idris et al., 2020; Kerimoglu et al., 2020). Both the cream, ointment, and emulgel have a homogeneous texture and no granules.

Based on organoleptic consistency, the texture from the thickest is ointment > emulgel > cream (Table II). These results are in line with the results of spreadability and stickiness in Table III, resulting in the higher viscosity, the smaller the spreadability value. These results occur because the ointment base is polyethylene glycol (PEG) 4000 which has a solid mass. It means that the higher concentration of PEG 4000 causes a higher ointment viscosity. The combination of PEG 4000 and PEG 400 is to lower the melting point of PEG 4000 to get an easier mixing process with other ingredients (Dewi et al., 2018). The PEG base was chosen because it is easily washed off with water, has a longer adhesion, is well distributed on the skin, and does not inhibit gas exchange and sweat production (Donkor et al., 2020). The properties of appearance, viscosity, and pH value in this result were similar to the previous results of the gel dosage form (Pratimasari et al., 2015).
Emulgel consists of emulsion and gel that facilitate topical delivery of lipophilic compounds, are easy to spread, easy to wash off, and are non-greasy, transparent, and in high demand (Phad et al., 2018). This gel form produces a thicker consistency, small spreadability, and long adhesion compared to creams (Tables II and III) because it contains polymers such as carbopol 940. This polymer has an acidic pH (pH 2-3) and will form a better gel if the pH increases along with the addition of triethanolamine in the formulation. The higher the carbopol, the higher triethanolamine is needed because the carbomer molecular bond increases and produces a thicker gel (Iceri et al., 2022).

The type of cream in this study is vanishing cream which is oil in water in a vaseline album base. This type of cream is suitable for delivering BEO topically on the skin (Robatjazi et al., 2022). If more vaseline album is used, the resulting cream will be thinner and easier to spread (Santoso et al., 2020). Although the BEO cream was thinner than the ointment and emulgel, the vaseline album content was enough to produce a spreadable layer between 5-7 cm (Table III) for ease of deployment.

The higher concentration of BEO, the shorter adhesion of the cream, ointment, and emulgel (Table III). This fact occurs because BEO has a low melting point (5°C), is liquid (density at 25°C of 0.913 g/cm³; viscosity of 10.29 mm²/s) at room temperature (Idris et al., 2020) and gives a thinner texture to the three topical preparations. Ointment had the highest adhesion compared to cream and emulgel (Table III). The higher adhesion reflects the longer duration of attachment to the skin. It means that the probability of active substances being released from the base will be greater and the ability to cause activity is higher (Maesaroh et al., 2020). In other word, higher adhesion means higher stickiness.

The pH value of BEO was 4 because it contains many unsaturated fatty acids such as linoleic acid and linolenic acid (Idris et al., 2020). This pH value causes the topical preparation value to be lower, that is 5-6 (Table III). The pH value is in accordance with the normal pH of the skin, which is between 4.5-6.5. Based on the pH value, it is safe and comfortable to use on the skin. If the pH of the topical preparation is too low, it will cause skin irritation while too high pH causes scaly skin (Maesaroh et al., 2020).

### Antibacterial activity

The antibacterial activity of BEO-containing cream, ointment, and emulgel is indicated by the diameter of the inhibition zone around the wells (in mm). The larger of a diameter of the inhibition zone, the stronger the antibacterial activity and the more potential to be developed into antibacterial agents (Hossain et al., 2022). The negative control had no antibacterial activity (Table IV) which means that neither the cream base, ointment, nor emulgel could inhibit the growth of *P. acnes*. The emulgel containing 5% BEO showed no activity against *P. acnes*, while the cream and ointment showed very weak activity. The antibacterial activity against *P. acnes* from strongest to weakest at 15% BEO concentration was (1) ointment, (2) cream, and (3) emulgel. These results indicate that antibacterial activity is influenced by the type of base and the ability of the active substance to diffuse from the base to the bacterial growth medium. The antibacterial activity of the 15% BEO emulgel and the positive control (Medi-Klin® gel clindamycin 1%) had a similar zone of inhibition because both of them used a gel base which is a polymer. Because of the different polarity of the active substance of BEO and base polymer in cream and emulgel, it makes a difficulty for the active substance to diffuse into the *P. acnes* growth medium to provide activity.
It is known that vaseline is a hydrophobic cream base (Varaka et al., 2022) which makes the hydrophobic BEO will be more retained in vaseline, resulting in the active substance that diffuses to the P. acnes growth medium being less. On the other hand, PEG is a hydrophilic ointment base (Madduru et al., 2020). It causes the hydrophobic BEO will be easily released from the ointment base and easy to diffuse to the P. acnes growth medium, resulting in the diameter of the inhibition zone of the ointment being larger than that of the cream. Moreover, the ointment has PEG which has a poly alcohol functional group. This chemical compound can make a hypertonic condition which helps bacteria difficult to grow.

This phenomenon indicated why F6 has stronger activity than F3 although they have the same BEO concentration (Table 4). On the other hand, F7 had no activity against P. acnes. If we analyze its formulation, F7 is an emulgel which is an emulsion that has surfactant to stabilize the emulsion system. BEO is a hydrophobic compound and has a high possibility of get strong interaction with this emulgel system. That is why the antibacterial activity of emulgel is lower than other dosage forms because it is difficult for BEO to enter the diffusion medium.

**CONCLUSION**

The resulted cream, ointment, and cream have a distinctive BEO aroma, are yellowish-white, and are homogeneous. Based on organoleptic consistency, the texture of the thicker and longer adhesion is ointment > emulgel > cream, respectively. The pH value of the topical preparation was 5-6 and easily leveled. Antibacterial activity against P. acnes from the strongest was ointment > cream > emulgel.

**REFERENCES**


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