

#### Effectiveness of Ethanol Gel and Essential Oil of Kencur Rhizome (Kaempferia galanga L.) base Karbopol against Propionibacterium acnes

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Abstract: Kencur rhizome (Kaempferia galanga L.) is an aromatic herbal plant as antibacterial and anti-inflammatory. This study aims to determine the physical quality and effectiveness of gel preparations of ethanol extract and essential oil of kencur rhizome based on carbopol inhibiting Propionibacterium acnes bacteria. This study used a Randomized Block Design (RAL) with 2 different treatments of 5 levels each, namely ethanol extract gel treatment and essential oil extract gel with concentrations of 10%, 20%, 30%, 40% and 50%. The results of the gel formulation of extracts and essential oils based on carbopol showed pH, texture, spreadability and adhesiveness according to the quality standards of topical preparations. The antibacterial inhibition of the gel was moderate at 50% extract concentration (11.41 mm); and 50% essential oil (9.04 mm). Positive control treatment inhibited growth by 15.84 mm while negative control showed no response. The extract gel and essential oil of galangal rhizome had a significant effect but were less effective in inhibiting P. acnes bacteria. Adding the maximum concentration of extract and essential oil can optimize acne healing.

Keywords: kencur rhizome; carbopol; gel; Propionibacterium acnes.

#### 1. INTRODUCTION

The face is the most important part for a person both women and men and everyone craves a white, clean face and free from facial problems, one of which is acne caused by bacteria Propionibacterium acnes (Anggraini et al., 2021). Acne is one of the common skin problems experienced by women and men in various ages (Dreno et al., 2018). The appearance of acne is characterized by the formation of inflammatory lesions, provoking chronic inflammation of polysebace. Although not classified as a chronic disease, acne greatly impacts the psychological life of sufferers (Dreno et al., 2019). Bacteria Propionibacterium acnes is the dominant cause of acne (Rasyid & Amody, 2020). Alternative acne treatment is usually with antibiotics. However, clinically inappropriate use causes side effects

that result in resistance and irritation in the long term (Afifi et al., 2018). Traditional medicinal plants and extracts are a good choice in inhibiting bacterial growth *P. acnes* shows results as a natural antibacterial ingredient (Alnabati et al., 2021).

Rimpang kencur (*Kaempferia galangal* L.) is an herbal medicinal plant of the family Zingiberaceae, having a high content of bioactivity used for the treatment of hypertension, chest and abdominal pain, toothache, rheumatism and dyspepsia (Subaryanti et al., 2022). The content of the compound *K. galangal* L. includes 2.5-4% essential oils, ethanol, methyl cinnamate, ethyl p-metoxynamate, pentadecan, borneol, camphene, kaempferal, kaempferida, p.metoccinnamic acid, and ethyl cinnamic (Khairullah et al., 2021). The high bioactivity content of ethyl-p-





methoxycinnamate of kencur rhizome plants is used as a sedative, anti-inflammatory effect, antiangiogenic and antibacterial activity (Kumar, 2020). Biosynthetic ethanol extract yields 95% of K. Galanga L. effectively inhibits bacteria Staphylococcus aureus (Samodra & Febrina, 2020). The balsamic aroma of kencur rhizomes is used as an ingredient *Reed Diffuser* Natural to improve sleep quality and minimize stressful conditions (Srivastava et al., 2019).

The content of essential oils, alkaloids, flavonoids owned by kencur rhizomes plays a role in antibacterial activity can be made comfortably and more practically as a gel preparation acne medicine (Silalahi, 2019). Gel is a topical preparation in accordance with physical quality standards for external use (Cendana et al., 2021). The formulation of gel preparations is composed of active substances, gelling agents and other ingredients (Agustiani et al., 2022). The concentration of gelling agents affects the properties and stability of absorption of the active substance of the gel on the skin (Putri et al., 2021). In this study used karbopol as a gel base, karbopol is often used for topical gel preparations. Carbopol is a non-toxic polymer and does not cause skin irritation. The advantages of using karbopol, can be developed in room temperature water with a viscosity range between 40,000 -60,000 cP (Safitri et al., 2021). The treatment of the carbopol base directly affects the viscosity properties of the gel preparation (Hari et al., 2018)

Material Active kencur rhizomes as antibacterial are obtained through a cold extraction process of 70% ethanol, producing ethanol compounds, essential oils, and EPMS (ethyl p-metoksisinamat). In addition to maceration, the essential oil of kencur rhizomes is extracted through the steam distillation process (Yao et al., 2018). The active ingredients in the study were ethanol and essential oils. Therefore, in this study researchers want to know quality physical preparations of carbopol-based gels, as well as seeing the effectiveness of active ingredients as an inhibitor of bacterial growth Propionibacterium acnes.

### 2. RESEARCH METHODOLOGY

#### **Research Design**

The research was conducted in January – March 2023 at the Microbiology Laboratory of the

Department of Biology FMIPA Cenderawasih University and the Pharmacy Laboratory of the Jayapura Poltekkes. This research method is an experimental method RAL (complete randomized design) 2 treatments of active ingredients of kencur rhizomes, namely ethanol extract and essential oil. The concentrations used were 10%, 20%, 30%, 40%, 50%, and clyndamycin positive control 1%.

## Isolation of ethanol and kencur essential oil (*Kaempferia galanga* L.)

Isolation of ethanol through the maceration method of simplisia ratio of 1: 4 with 70% ethanol solvent for 3x24 hours. The maceration results are filtered repeating 3 times to obtain filtrate. Isolation of essential oils through the steam distillation method, fresh rhizomes samples, cut into pieces and evaporated for 5 hours. The result of distillate is in the form of water and pure essential oil kencur. The active ingredient treatment was taken as much as 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml made concentrations of 10%, 20%, 30%, 40%, and 50% using 5% DMSO solvent as much as 10 ml.

#### **Gel Formulation**

Gel formulation of carbopol base 0.5%; 1%; and 2% are presented in Table 1. Karbopol is developed in a mortar, given hot water until a gel mass is formed. The addition of TEA, glycerin, propylene glycol and methyl paraben as appropriate, stirred until homogeneous and clear colored gel. Distilled water is added until homogeneous and a gel base is obtained. The addition of ethanol extract or essential oil according to each treatment concentration.

| <b>Table 1.</b> Formulation of Kencur Rhizome |  |
|---|--|
| Extract and Essential Oil Gel Prenarations    |  |

|                  | % b/b |       |       |  |  |
|------------------|-------|-------|-------|--|--|
| Material         | BE    | F II  | F III |  |  |
| Carbopol         | 0,5   | 1     | 1     |  |  |
| Gliserin         | 5     | 5     | 5     |  |  |
| Propyleneglycole | 10    | 10    | 10    |  |  |
| TEA              | 1     | 1     | 1     |  |  |
| Methyl paraben   | 0,1   | 0,1   | 0,1   |  |  |
| Air suling       | 50 ml | 50 ml | 50 ml |  |  |

(Suppose et al., 2016)





### Evaluation of gel preparations

#### Organoleptic

Organoleptic examination of ethanol extract gel and essential oil of kencur rhizomes was observed changes in taste, color, and smell.

#### Homegenitas

Homogeneity testing is done by applying 0.1 grams of gel on a piece of glass, the gel shows a homogeneous arrangement, and no coarse grains are visible.

#### pН

Determination of pH by dissolving a gel preparation of 100 mg, stirring until homogeneous and measuring universal pH.

#### Spread Force

A sample of 1 g of preparation, placed in the center of the two watch mirrors, the top glass is loaded sequentially starting from no load until the load weight reaches 150 grams.

#### Sticky Force

The adhesion check is carried out by placing the preparation on the glass of the object, the dosage part is covered with the glass of another object and pressed using a load of 1 kg for 5 minutes. The load is released, and a long time attached to the gel is recorded.

#### Antibacterial Activity Test of Ethanol Extract and Essential Oil of Kencur Rhizome

Testing the inhibitory power of bacteria using the agar diffusion method. First, a 100 mg gel preparation of each concentration is prepared, dissolved with 1 ml of aquadest. A blank disk is inserted and immersed into a solution of an extract gel preparation or essential oil. The blank disk is taken and placed on *MHA* (Muller Hinton Agar) media containing *P. acnes* bacteria. The test media was incubated at  $37^{\circ}$ C for 24-48 hours. Measurement of the diameter of the clear zone formed around the blank disk using a caliper.

#### **Data Analysis**

Research data in the form of gel evaluation results were analyzed in a qualitative descriptive manner. The inhibitory power observation data was followed by a one-way variance analysis test (ANOVA) with a confidence level of 5%. If the effect is significant, then continue the *Smallest Real Difference* test to see the difference between each treatment.

#### 3. **RESULTS AND DISCUSSION** Ethanol and Essential Oil Extraction *of K. galanga* L.

The extraction results of this study used the parameter percent yield, maceration extraction simplisia 500 grams with ethanol 70% ratio 1: 4 produced a thick extract of 46 grams (9.2% yield). The steam distillation of 250 grams of kencur rhizomes produced 30 grams of pure essential nyak noodles (Table 2).

**Table 2.** Ethanol Extract and Essential Oil Yield

 K
 galangal L

| K. galangal L. |                 |                       |                   |                    |  |  |  |
|----------------|-----------------|-----------------------|-------------------|--------------------|--|--|--|
| No             | Observatio<br>n | Berat<br>Simplis<br>a | Weight<br>Extract | %<br>Rendem<br>ing |  |  |  |
| 1              | Ethanol         | 500                   | 46                | 9.2                |  |  |  |
| 1              | Lunanoi         | grams                 | grams             | ),2                |  |  |  |
| 2              | Essential oil   | 250                   | 30                | 12                 |  |  |  |
| 2              | Essential OII   | grams                 | grams             | 12                 |  |  |  |

As shown in Table 2 data, for the ethanol extraction process produces a yield of 9.2%, simplisia macerated for 3x24 hours with 2xrepetition of remaceration. A good extract of kencur is not less than 8.3% and essential oil is not less than 2.4% w/b (Kemenkes RI, 2017). A high percentage of yield indicates the presence of active substance compounds contained in extracts or essential oils is quite large, so that the greater the amount of yield obtained, the more components of active substance compounds extracted. Isolation of essential oils is more widely obtained in young plants (9-12 months) in the organs of leaves, fruits and seeds (Arsa &; Achmad, 2020). 70% ethanol is an extract solvent K. galangal L. is polar, helping to attract flavonoid compounds and alkaloids in polar solvents (New et al., 2022).

The mechanism of action of maceration is easier and the tools are simple. The duration of the extraction process time affects the interaction between solvent and simplisia, the longer the extraction time allows the process of synthesis of essential oils from plant tissues to be extracted more (Febriyanto, 2022).

# Physical Quality of Gel Extract and Essential Oil *K. galanga* L.

Physical quality evaluation of gel preparations aims to determine the physical properties of the preparation and compare the





physical quality of the preparation whether it has met the standard requirements according to the established criteria.

Results of evaluation of extract gel and essential oil of kencur rhizomes (*K. galangal* L.) indicates that the physical gel is in accordance with the quality standards of topical preparations. The addition of karbopol as a gelling agent is hydrophilic. The properties of carbopol make it easy to attract flavonoid compounds and alkaloids produced by kencur extract, increase the consistency of the gel, easily dispersed in water (Alvionida et al., 2021).

Gel, extract and essential oil of rhizomes of kencur are considered good and can be applied to the skin (Hasanah & Novian, 2020). The formulation of the addition of propylene glycol is consolen, being a good solvent for kencur rhizomes, has an anti-inflammatory role. EPMS (ethyl p-metoxycinamate) is an important compound in kencur rhizomes. On research <u>Wahyuni et al. (2022)</u>, antiinflammatory activity synthesized from ethanol of kencur rhizomes has an effect on wound healing, this is due to the activity of EPMS compounds of kencur rhizomes. EPMS is classified as a polar carbonyl group, acting as a solvent of active ingredients in the form of extracts and homogeneous essential oils to form a gel (<u>Primawati & Jannah, 2019</u>). Additional formulations of triethanolamine (TEA) also play a role in stabilizing the pH of the gel, TEA contains organic chemical components of clusters.

The gel formulation uses karbopol as a gel base with a concentration of 1%. The following is the result of the evaluation of the active ingredient gel of ethanol extract and essential oil of kencur rhizome (*K. galanga* L.) with 5 different concentrations, including F1 (10%), F2 (20%), F3 (30%), F4 (40%), and F5 (50%). The evaluation results can be seen in Tables 3, 4 and graphs 1 and 2 below

**Table 3.** Results of Organoleptic Examination of Gel Extract and Essential Oil of Kencur Rhizome

 (K. Galanga L.)

| No.  | Observation |        | Organoleptic average yield |                |                |                |                |
|------|-------------|--------|----------------------------|----------------|----------------|----------------|----------------|
| 140. | Obser       | vation | <b>F1</b>                  | F2             | F3             | F4             | F5             |
| 1.   | Extract     | Smell  | typical kencur             | typical kencur | typical kencur | typical kencur | typical kencur |
|      |             | Color  | Chocolate                  | Chocolate      | Chocolate      | Chocolate      | Coklat         |
|      |             | Shape  | Gel                        | Gel            | Gel            | Gel            | Gel            |
| 2.   | Essential   | Smell  | typical kencur             | typical kencur | typical kencur | typical kencur | typical kencur |
|      | oil         | Color  | Pale yellow                | Pale yellow    | Pale yellow    | Pale yellow    | Pale yellow    |
|      |             | Shape  | Gel                        | Gel            | Gel            | Gel            | Gel            |

Table 3 with formulation 5 concentrations of extracts and essential oils differ, each of which produces a gel-shaped dosage form. The color produced by ethanol extract gel preparations and essential oils is different, the color of extract gel preparations is brown, for essential oils produce a pale-yellow gel color. The smell of gel preparations in formulations F1, F2, F3, F4 and F5 does not change, the resulting odor is a characteristic smell of kencur.

Based on the results of organoleptic observations of gel preparations of rhizomes kencur on. Essential oils, good gel preparation parameters, namely having a semi-solid form (gel), clear white if without extract or colored like extract, odorless without extract or characteristic smell of the extract used and have a cold sensation when applied to the skin (Sari et al., 2022).

In addition, measurements of the level of preference for the preparation are also carried out. This favorability level is made with a scale: very like, like, dislike and very dislike. In this test there were 30 panelists and asked to rate the preparation based on the shape, smell, and color of the gel preparation.





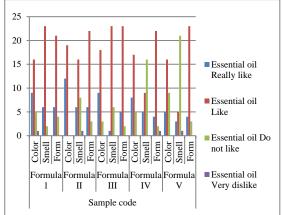


Figure 1. Test results of respondents' favorability of organoleptic tests of essential oil preparations of kencur rhizomes

Based on the results of the favorability test, it showed that panelists liked kencur essential oil gel preparations at F1 (10%), F2 (20%) and F3 (30%), while at F4 (40%) and F5 (50%) panelists chose Dislike of smell, but for color and shape, panelists chose Like the most.

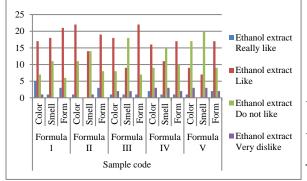


Figure 2. Test results of respondents' favorability of organoleptic test of ethanol extract gel preparation of kencur rhizomes

Based on the results of the favorability test, it was shown that panelists liked the most gel preparations of ethanol extract f1 (10%) and F2 (20%), for F3 (30%) and F4 (40%) panelists liked the color and shape but did not like the smell of the kencur ethanol extract gel preparation, and for F5 (50%) panelists liked the shape but did not like the color and smell of the kencur ethanol extract gel preparation.

**Table 4.** Results of Homogeneity Examination of

 Gel Extract and Essential Oil of Kencur Rhizome

|     | (K. galangal L.)       |                              |                 |                 |                 |                 |  |  |
|-----|------------------------|------------------------------|-----------------|-----------------|-----------------|-----------------|--|--|
| No  | Obser                  | Average yield of homogeneity |                 |                 |                 |                 |  |  |
| 140 | vation                 | F1                           | F2              | F3              | F4              | F5              |  |  |
| 1   | Ethano<br>l<br>extract | Homog<br>eneous              | Homog<br>eneous | Homog<br>eneous | Homog<br>eneous | Homog<br>eneous |  |  |
| 2   | Essenti<br>al oil      | Homog<br>eneous              | Homog<br>eneous | Homog<br>eneous | Homog<br>eneous | Homog<br>eneous |  |  |

Based on the results of the homogeneity test of the preparation in Table 4, the five formulations of the extract gel preparation and essential oil of kencur rhizomes do not show coarse grains, the gel has a homogeneous arrangement of particles characterized by all parts mixed with. All gel preparations have good homogeneity, so they are considered eligible according to topical preparations.

SNI 06-2588 standard is characterized by a homogeneous gel arrangement based on a perfectly even color equation and no coarse particles or lumps of gel additives are visible when rubbed and overlapped with a glass plate (Julianti et al., 2023).

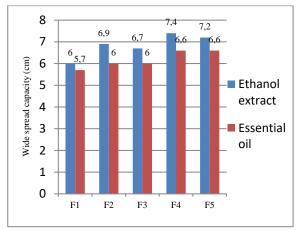
| Table 5. pH Examination Results of Gel Extract |
|--|
| and Essential Oil of Kencur Rhizome (K.        |

| galanga L.) |    |               |    |    |    |           |  |
|-------------|----|---------------|----|----|----|-----------|--|
| No          | L  | - Information |    |    |    |           |  |
| INO         | F1 | F2            | F3 | F4 | F5 | mormation |  |
| 1           | 6  | 6             | 6  | 6  | 6  | Qualify   |  |
| 2           | 6  | 6             | 6  | 6  | 6  | Qualify   |  |

The pH evaluation results of both gel preparations with 5 different formula concentrations (Table 5) showed a stable pH value of 6. Antiacne gels formulated from extracts and essential oils of kencur rhizomes are considered qualified as gel preparations. SNI Standard 06-2588 pH value of gel preparations according to SNI Standard 06-2588, namely the physiological pH of facial skin ranges from 4.5-6.5 (Rohman et al., 2020). If the pH is too acidic it will cause irritation and inflammation causing acne and if the pH is too alkaline it will cause dry, scaly and sensitive skin (Chilicka et al., 2021).

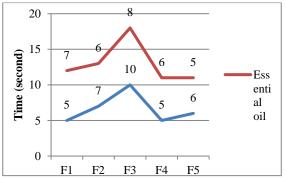






**Figure 3.** Wide Dispersion Graph of Gel Extract and Essential Oil of Rhizome of Kencur (*K. galanga* L.)

Based on Figure 3 shows the extent of dispersion of gel preparations using extract and essential oil formulations. Gel preparations at concentrations of F1, F2 and F3 the graph is unstable. At concentrations of F2 (20%) and F4 (40%) the dispersion value increased, F3 (30%) and F5 (50%) formulations increased. For gel preparation formulations using essential oils, the graph has a steady increase, the wide range of dispersion values is 5.7 - 6.6 cm. SNI standard 06-2588 values the dispersion power of good gel preparations, which have a spreading area ranging from 5-7 cm (Julianti et al., 2023). Gel formulations of extract gel preparations at concentrations of F4 and F5 are considered not to meet the criteria as gels, dispersion values of more than 7 cm. The formulation of gel preparations \_ with essential oil ingredients is considered good so that it meets the criteria as a gel.



**Figure 4.** Graph of Long Time of Adhesion of Gel Extract and Essential Oil of Kencur Rhizome (*K. galangal* L.)

Based on Figure 4, the graph shows the length of time the adhesion of extract gel preparations and essential oils has increased and decreased. Formulations of extracts and essential oils at concentrations of F1 (10%), F2 (20%), F4 (40%), and F5 (50%) showed an average duration of stable gel preparations, which was a time span of 5-7 seconds. For adhesion of gel preparation formulations at a concentration of F3 (30%) obtained a long duration of stickiness, which is 8-10 seconds. The higher the concentration of the carbopol gel base (concentration 1%), the thicker the consistency of the preparation and the greater or longer the adhesion value (Irianto et al., 2020). The standard adhesion requirements set on good gel preparations are to have a adhesion time of more than 1 second (Lewa et al., 2023). Of the five gel preparation formulations considered to meet the criteria for qualification as a gel, the length of adhesion time of a gel preparation is > 1 second.

**Table 6.** Gel Inhibitory Zone of Extract andEssential Oil of Kencur Rhizome (K. galanga L.)

| against P. acnes          |                                       |                                   |                                     |                                     |  |  |  |
|---------------------------|---------------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|--|--|--|
| Treatment                 | Average<br>(mm)<br>Ethanol<br>extract | Average<br>BNT<br>0.01<br>Ethanol | Average<br>(mm)<br>Essential<br>oil | Average<br>BNT<br>0.01<br>Essential |  |  |  |
|                           |                                       | extract                           |                                     | oil                                 |  |  |  |
| Control (-)               | 0                                     | 0                                 | 0                                   | 0                                   |  |  |  |
| Check(+)                  | 15,98d                                | 17,22                             | 15,84d                              | 16,8                                |  |  |  |
| F1 (10%)                  | 6,35a                                 | 7,59                              | 5,58a                               | 6,54                                |  |  |  |
| F2 (20%)                  | 8,11b                                 | 9,35                              | 6,65b                               | 7,61                                |  |  |  |
| F3 (30%)                  | 8,15b                                 | 9,39                              | 7,06b                               | 8,02                                |  |  |  |
| F4 (40%)                  | 9,52c                                 | 10,76                             | 7,26b                               | 8,22                                |  |  |  |
| F5 (50%)                  | 10,14c                                | 11,38                             | 9,04c                               | 10,00                               |  |  |  |
| BNT 1% ethar              | nol extract                           | 1,24                              |                                     |                                     |  |  |  |
| BNT 1% Essential oil 0.96 |                                       |                                   |                                     |                                     |  |  |  |
|                           |                                       |                                   |                                     |                                     |  |  |  |

Based on the results of observations of the average inhibitory diameter in Table 6 shows that the effect of ethanol extract gel and essential oil is significant on the growth of *P. acnes bacteria*. A treatment concentration of 50% is considered quite effective in inhibiting bacteria, the average diameter of the inhibitory zone  $\leq 20$  mm (Table 6).

Strong inhibition is shown by the positive control treatment of clyndamycin 1%, the diameter of the clear zone is not much different from the treatment of gel preparations. Research by Maria (2014) proved that ethanol extract of kencur rhizomes effectively inhibits bacterial





growth *Salmonella typhi* at a concentration of 90-100%. Bakteri *P. acnes* including the type of Gram positive bacteria, the cell wall is composed of 2 layers, namely the cytoplasmic membrane and the thick peptidoglycan layer (Rostikawati et al., 2021). When compared to bacteria *Salmonella* is a bacterium Gram negative, its cell wall layer is composed of a thin peptidoglycan layer. The structure of the peptidoglycan layer affects the absorption of gel preparations. Inhibition zone against *Salmonella* higher compared to *P. acnes*.

Kencur rhizomes are indicated to contain flavonoids and alkaloids, able to inhibit bacterial growth. Flavonoids play a role in inhibiting nucleic acid synthesis, membrane function and bacterial cell metabolism (Wang et al., 2021). The mechanism of action of alkaloids as antibacterials, damaging the peptidoglycan layer that makes up the bacterial cell wall. If the cell wall layer cannot be formed intact, it causes death in the bacterial cell. The antibacterial effect caused may come from the activity of secondary metabolite compounds of kencur rhizomes (*K. galangal* L.) (Song et al., 2021).

#### 4. CONCLUSION

Evaluation of the stability of gel preparations, active ingredients, ethanol extract and essential oil of rhizomes of kencur (K. galanga L.) in accordance with the physical quality of topical dosage requirements. The concentration of the gel has a significant effect on inhibiting the growth of *Propionibacterium acnes* bacteria in the 50% formula, but it has not been effective as an antibacterial.

Further research is needed to see the amount of concentration of secondary metabolite compounds of kencur rhizomes (*Kaempferia galanga* L.) which is more optimal in inhibiting bacteria, the content of these chemical compounds can be developed to innovate natural beauty products.

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