

## ANTIBACTERIAL ACTIVITY OF JAVANESE CARDAMOMUM FRUIT ESSENTIAL OIL (*Wurfbainia compacta*) ON *Propionibacterium acnes*

Ali Nofriyaldi<sup>1\*</sup>, Ayu Rahmawati<sup>1</sup>, Sri Rezeki Nur Endah<sup>1</sup>

<sup>1</sup>Department of Pharmacy, Faculty of Health Science, Perjuangan University of Tasikmalaya, Indonesia

\*Email Corresponding: [alinofriyaldi13@gmail.com](mailto:alinofriyaldi13@gmail.com)

### ABSTRACT

Cardamom fruit contains the essential oils cineol, terpineol and borneol which have the potential to act as antibacterials against the growth of *Propionibacterium acnes*. This study aimed to determine the percentage yield of essential oil and the value of the inhibition zone for the antibacterial activity of cardamom fruit oil. The research method begins with the preparation of dried simplicia from cardamom fruit, which is then distilled using water steam. The essential oil obtained was subjected to antibacterial activity using the well diffusion method. The results of the research showed that the yield of cardamom fruit essential oil was 1,66%. Cardamom fruit simple powder contains secondary metabolite compounds alkaloids, flavonoids, saponins, steroids, triterpenoids and polyphenols. The antibacterial activity test against *Propionibacterium acnes* at concentrations of 3%, 6%, and 12% produced inhibition zones of 3.2 mm, 4.7 mm, and 10.1 mm, respectively. Therefore, it can be concluded that at concentrations of 3% and 6%, it is included in the medium category, while at a concentration of 12%, it is included in the strong category.

**Keywords:** Antibacterial, Oil of Cardamomum Fruit, *Propionibacterium acnes*, *Wurfbainia compacta*

### INTRODUCTION

Acne is a skin problem, especially on the face, and can interfere with physical appearance. The prevalence of acne cases increases every year, especially in teenagers, at 85% (Sirajudin et al., 2019). The cause of acne is the formation of nodules, pustules, blackheads, and papules that grow in the facial area, which usually occurs in teenagers (Malfasari, 2017), and also irregular hormonal factors, especially in teenagers. In addition, acne can be caused by the face producing excess oil glands, causing skin infections. Skin infections on the face are caused by several bacteria, including *Propionibacterium acnes*. This bacteria plays an important role in the pathogenesis of acne vulgaris into acne because it can produce lipase which breaks down free fatty acids from skin lipids (Zahrah et al., 2018). There are many treatments for acne on the market, such as topical and oral antibiotics clindamycin, topical and oral erythromycin, and oral doxycycline. However, long-term use may increase the risk of side effects. The prevention of these side effects can be achieved by using active ingredients based on natural ingredients, especially plants of local origin, such as Javanese cardamom (*Wurfbainia compacta*).

Javanese cardamom (*Wurfbainia compacta* Sol. Ex Maton) is a plant native to Indonesia, a species of cardamom with local wisdom value originating from West Java, and has potential as a medicinal plant (Nofriyaldi et al., 2023). It is generally used as a spice because cardamom contains many essential oils and secondary metabolite compounds (Silalahi, 2017).



**Figure 1. Cardamom Plant and Cardamom Fruit**

The active compounds that have been confirmed in cardamom plants are cineol, metal hepton,  $\beta$ -terpeniol, sabinen, linalool, geraniol,  $\alpha$ -pinene, sabinen, limonene, terpenyl acetate, saponins, flavonoids, polyphenol compounds, starch, sugar, fat, proteins, and silicates (Winarsi, 2014). One of the studies related to the yield of cardamom fruit oil was by steam distillation, where previous research had carried out making cream preparations using cardamom oil as the active ingredient. The results showed that the inhibition zone for the growth of *Staphylococcus aureus* was 10,883 mm at a concentration of 15%, which was included in the strong category (Anugrah et al., 2018). Antibacterial activity of the ethanol extract of cardamom fruit against *p. acnes* produced an inhibition zone of 21.88 mm at a concentration of 30%, which is considered very strong (Nofriyaldi et al., 2023). However, no research has been conducted to test the activity of cardamom oil against *Propionibacterium acnes*.

## RESEARCH METHODS

### Tools and materials

**Tools :** Autoclave (GEA), stirring rod (ROFA), beaker glass (Iwaki®), blender (Maksindo), petri dish (Normax), porcelain cup (ROFA), glass funnel (Iwaki®), measuring cup (Pyrex®), hot plate (18-One), vernier calliper (Vernier Calliper), laminary air flow (Monmouth), incubator (18-One), ose, pH meter (ATC), test tube (Iwaki®), Analytical ballance (Newtech), vacuum rotary evaporator (eyela).

**Materials:** Javanese cardamom extract (*Wurfbainia compacta*), citric acid (Merck), distilled water (Water one), *Propionibacterium acnes*, EDTA (Titriplex), NaCl (Emsure), ethanol 96% (Volenvi), kassa, Potato Dextrose Agar (PDA) (Millipore), propylene glycol (Dow USP), sodium lauryl sulfate (Texapon BASF), and oleum rosae (aloin).

### Research Procedure

#### 1) Material collection

Javanese Cardamom (*Wurfbainia compacta*) fruit was obtained in Sukamulih Village, Sariwangi District, Tasikmalaya Regency, West Java Province.

#### 2) Plant Determination

Cardamom plants were used as research samples and were obtained from the Laboratory of the School of Biological Sciences and Technology, ITB. The determination results confirmed that the plant specimen was indeed a Javanese Cardamom plant (*Wurfbainia compacta* Sol. Ex Maton) with No. 5089/IT1.C11.2/T.A.00/2023.

#### 3) Processing of Test Materials

Samples of Cardamom Fruit (*Wurfbainia compacta*) were collected, washed, or wet sorted using running water, and then chopped and air-dried. After drying, dry sorting was performed, followed by crushing using a blender and sieving using a number 40 sieve (Nofriyaldi and Agustien, 2020a).

#### 4) Phytochemical Screening of Dried Simplicia Cardamom Fruit (*Wurfbainia compacta*)

Cardamom fruit simplicia undergoes phytochemical screening, including testing for the content of polyphenols, alkaloids, flavonoids, saponins, steroids, and triterpenoids (Sukandar et al., 2015).

5) Steam Distillation of Cardamom Fruit

Cardamom fruit (50 g) from the sieve was placed into a steam and water distillation apparatus for 6 hours with a sample and water ratio of 1:8. The oil obtained was collected into a chemical glass, separated using a separating funnel, stored in a vial, and the yield level was calculated (Tambunan, 2017).

6) Test the Antibacterial Activity of Cardamom Fruit Oil

6.1. Tool Sterilization

The tools used in this study were washed and sterilized by autoclaving at a temperature of 121 °C for 15 minutes.

6.2. Making Nutrient Agar (NA) Media

A total of 7.25 grams of nutrient agar was weighed and dissolved in 250 ml of distilled water, then placed into an Erlenmeyer flask and heated until the NA dissolved. The homogenized media was sterilized in an autoclave for 15 minutes at a temperature of 121°C, and the media was allowed to cool to approximately 40°C - 45°C, the cooled media was poured into a petri dish and allowed to solidify (Dajoh et al., 2020).

6.3. Preparation of McFarland Solution Turbidity Standard

A total of 9.95 ml of 1% H<sub>2</sub>SO<sub>4</sub> solution was mixed with 0.5 mL of 1.175% BaCl<sub>2</sub>.2H<sub>2</sub>O solution in an Erlenmeyer flask. The solution was then shaken until a cloudy solution was obtained (Wardaniati and Gusmawarni, 2021).

6.4. Making Test Solutions

Cardamom fruit oil test samples were prepared at concentrations of 3%, 6%, and 12%. Clindamycin was used as a positive control, while PEG 400 solution was used as a negative control (Wirawan et al., 2018).

6.5. Preparation of *Propionibacterium acnes* Bacterial Suspension

*Propionibacterium acnes* bacterial suspension was prepared by culturing *Propionibacterium acnes* bacteria using a sterile wire, and then suspended in a test tube containing 10 ml of 0.9% NaCl until the same turbidity as the McFarland solution turbidity standard was obtained.

6.6. Antibacterial Activity Testing

Antibacterial activity testing was performed using the well method. 3 holes were made in the media for the samples to be tested in 5 Petri dishes, namely the positive control, negative control, and cardamom fruit oil with concentrations of 3%, 6%, and 12%. The test samples were left to absorb into the media and incubated at 37 °C for 24 hours, and the diameter of the inhibition power (mm) was measured using a caliper on each sample. Clindamycin gel (1%) was used as the positive control, while PEG 400 was used as the negative control (Saadah et al., 2020).

## RESULTS AND DISCUSSION

### Destillation

Cardamom oil was obtained by steam distillation at a ratio of 1:8. The distillation method using water-steam distillation (water and steam distillation) has the advantage that it only requires water, and the equipment is simple but can produce essential oils. Cardamom fruit simplicia powder (700 g) was distilled in 5.6 liters of distilled water at a ratio of 1:8. Because the solvent (water) is in sufficient quantity, temperature and pressure can be controlled more effectively during the distillation process. This avoids damage to the plant material that can occur if it is too concentrated, and optimizes extraction with the ratio, providing enough space for the steam formed during the distillation process. Water helps lift essential oils from plant materials, and sufficient water helps keep the temperature from getting too high, so that essential oil extraction can be done more effectively and efficiently. Increasing the Extraction yield increases the amount of vapor formed and makes it easier to separate the essential oils from water. Steam availability the distillation process relies on steam to lift oils from plant material. If there is too little solvent, the steam produced can be limited, reducing the efficiency of the distillation process. The distillation of 700 grams of cardamom fruit produced 11.5908 grams of cardamom fruit oil with a yield of 1.66%.



**Figure 2. Results of Destilation From Essential Oil Fruit Cardamomum**

**Table I. Results of Destilation**

<b>Cardamomum Fruit</b>	<b>Results</b>
Distilled cardamom fruit simplicia powder	700 gram
Cardamom fruit oil	11,5908 grams
Cardamom fruit oil yield	1,66%

The results of the distillation did not meet the standards, namely 2-5% of the dry simplicia, because when the drying process takes too long, some of the essential oil content is lost or evaporates. The distillation time was not optimized because it used the general time, namely 5 hours, so the essential oil was not completely extracted. The particle size was suboptimal in the distillation process, so the essential oil extraction process was not efficient ([Amaliah et al., 2022](#); [Elisa Loppies et al., 2021](#); [Irfan et al., 2022](#)).

### **Phytochemical Screening**

Phytochemical screening of cardamom fruit powder revealed the presence of saponins, flavonoids, alkaloids, polyphenols, steroids, and triterpenoids. In the polyphenol compound test, positive results were obtained, indicated by the formation of a blackish-green color. This color change occurs due to the formation of a complex compound between polyphenols and  $\text{FeCl}_3$  ([Ikalinus et al., 2015](#)). The alkaloid compounds of cardamom fruit powder were tested using three reagents, and positive results were indicated by the presence of sediment or color changes. Testing the saponin compound of cardamom fruit powder showed positive results, marked by the formation of foam. According to [Nurzaman et al. \(2018\)](#), the formation of foam in saponin compounds is due to the reduction of water surface tension and the presence of glycosides, which hydrolyze into glucose and form foam on the water surface after shaking. In testing the flavonoid compounds in cardamom fruit powder, positive results were obtained, which showed that the powder had formed a yellow color. This color change occurs because flavonoid compounds can be reduced with  $\text{Mg}$  and  $\text{HCl}$ , which can produce red, yellow, or orange colors ([Yeni Aprilia et al., 2022](#)). In testing steroid and triterpenoid compounds, cardamom fruit powder showed positive for triterpenoids with the formation of a deep purple color. This color change is based on the ability of triterpenoid compounds to form a color with sulfuric acid in anhydrous acetic acid ([Ikhwan Habibi et al., 2018](#)).

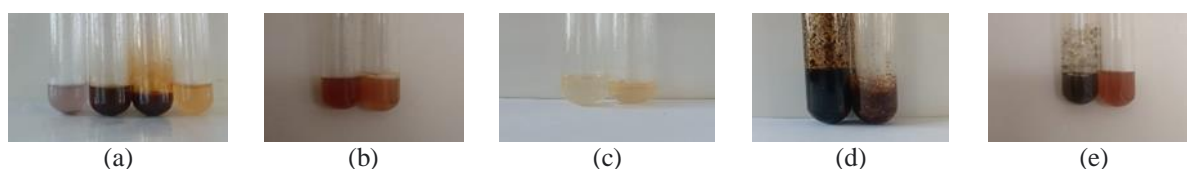


**Table II. Results of Phytochemical Screening**

Examination	Results	Reagent	Characteristics
Alkaloids	+	Mayers Dragendorfs	A white precipitate is formed A yellow precipitate is formed
Saponins	+	Distilled water, Dilute HCl	Foam is Formed
Flavonoids	+	Mg, Dilute HCl and amyl alcohol	A red color is formed which can be attracted by amyl alcohol
Steroids and Triterpenoids	+	Chloroform, anhydrous acetic acid and H <sub>2</sub> SO <sub>4</sub>	A purple color is formed
Polyphenols	+	FeCl <sub>3</sub>	A blackish green color forms

Notes :

(+) The presence of a class of secondary metabolite compounds is detected



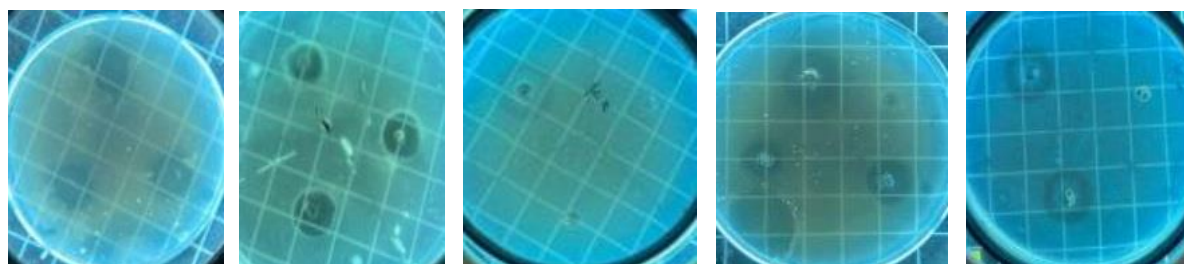
**Figure 3. Results of Alkaloids (a), saponins (b), flavonoids (c), Steroids and Triterpenoids (d), and polyphenols (e).**

#### Test of the Antibacterial Inhibitory Activity of Cardamom Essential Oil

Testing the antibacterial activity of cardamom fruit oil using nutrient agar media. The use of nutrient media is carried out with the aim of growing *Propionibacterium acnes* because this media has many sources of nitrogen, carbon, and vitamins.

**Table III. Antibacterial Test Results**

Sample	Average
Control (+)	11,75 mm $\pm$ 0,02
Control (-)	7,3 mm $\pm$ 0,01
Formula I 3%	3,2 mm $\pm$ 0,04
Formula II 6%	4,7 mm $\pm$ 0,01
Formula III 12%	10,1 mm $\pm$ 0,02



**Figure 4. Results of antibacterial From Positive Control, Negative Control, 3%, 6%, and 12%**

Based on these results, cardamom fruit oil with varying concentrations of 3%, 6%, and 12% can inhibit the growth of *Propionibacterium acnes* bacteria because there is a clear zone around the well. This antibacterial activity test used clindamycin as a positive control because it can reduce the

concentration of *Propionibacterium acnes* and inflammatory mediators, which is indicated for the treatment of mild and moderate acne (Sirajudin et al., 2019).

In this study, there were differences in the results of the antibacterial activity test, which showed that the greater the concentration of the extract used, the greater the clear zone formed, because the higher the concentration of the extract used, the more active substances contained to inhibit bacteria increase in area. At low concentrations, cardamom fruit oil contains few antimicrobial substances, so the inhibitory power or clear zone formed is reduced. Oily substances are difficult to penetrate in antibacterial testing using the well method on Nutrient Agar media because oil is hydrophobic (does not dissolve in water), while Nutrient Agar media are water-based. Due to this difference in polarity, oil tends to float or form droplets on the surface of the media rather than spreading into it. In addition, oil has a higher viscosity than water-based solutions, making it difficult to penetrate wells and spread evenly in the media. Another reason related to the rate of diffusion is that water-soluble antibacterial substances can diffuse easily through agar media, while oily substances have difficulty moving from the well to the area around the bacteria being tested; therefore, their antibacterial activity is less than optimal. Therefore, to increase the diffusion rate of cardamom oil, it is necessary to add substances that can be used in the manufacture of pharmaceutical preparations, so that the size of the inhibition zone can be increased (Nurhikma et al., 2024). By formulating cardamom oil in a semi-solid preparation, it is hoped that it will affect the biopharmaceuticals that occur, such as extending contact time, preventing evaporation of essential oils, and reducing the risk of irritation, which increases the effectiveness of active compounds, safety, and comfort (Hadiq et al., 2024).

In the test, the negative control produced an inhibition zone of 7.3 mm, which is categorized as moderate because when PEG is applied to the bacterial environment, it can cause osmotic conditions that are unfavorable for the bacteria (Fatimah et al., 2016). This can cause dehydration of bacterial cells, which ultimately inhibits their growth or even kills them. Bacteria such as *P. acnes* are sensitive to changes in osmotic pressure, and fluid imbalance can damage the bacterial cell structure. In addition, PEG can disrupt the integrity of cell membranes by reducing lipid solubility or modifying membrane function (Fatimah et al., 2021). This can cause the leakage of important cellular components, ultimately inhibiting bacterial growth or function. Another reason is that PEG can reduce the activity of bacteria in producing these fatty acids, which, in turn, reduces inflammation and prevents acne formation.

## CONCLUSION

Cardamom Fruit Oil (*Amomum compactum* Sol. Ex Maton) has inhibitory activity against the growth of *Propionibacterium acnes* bacteria where a concentration of 12% shows the most effective results with an average inhibitory zone diameter of 10.11 mm which is in the strong category. It can be concluded that the higher the concentration of cardamom fruit extract, the greater the diameter of the inhibition zone against *Propionibacterium acnes*. This concentration can be used as a basis for making a formulation that is more practical to use, such as a cream or gel preparation, and it is necessary to test antibacterial activity using other methods.

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