# TRACING SECONDARY METABOLITES AND ANTIBACTERIAL ACTIVITY ETHANOL EXTRACT OF LAKUM LEAF (*CAYRATIA TRIFOLIA* L. DOMIN), AGAINST ACNE-CAUSING BACTERIA (*PROPIONIBACTERIUM ACNE DAN STAPHYLOCOCCUS EPIDERMIDIS*)

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**Abstract:** Acne is one of the most common skin diseases suffered by humans. Antibiotics are the first line of acne treatment therapy, but the use of antibiotics needs to be reviewed because of cases of resistance. Lakum leaves (Cayaria trifolia L. Domin) is a plant that contains chemical compounds that can be used as alternative therapies, including flavonoids, alkaloids, tannins and saponins that function as antibacterial. The purpose of this study was to determine the role of lakum leaf (Cayratia trifolia L. Domin) as a natural antibacterial for acne therapy. This research is a quantitative study using the true experimental design method, which is measuring the clear zone of lakum leaf extract against Propionibacterium acnes and Staphylococcus epidermidis bacteria found in Nutrient Agar (NA). The antibacterial test method in this study used the well method with variations in the ethanol extract of lakum leaves (Cayratia trifolia L. Domin) with 4 concentrations, namely 25%, 50%, 75% and 100% concentration, acetone as a negative control and clindamycin 30 ppm as positive control. The results showed that the ethanol extract of lakum leaves (Cayratia trifolia L. Domin) contained alkaloids, flavonoids, tannins and saponins. The greatest concentration obtained in inhibiting bacterial growth was at a concentration of 100% extract with an average zone diameter of 20.5 mm for P. acnes bacteria and 21.5 mm for S. epidermidis bacteria.

**Keywords:** Antibacterial, cayratia trifolia, P. acne, S. epidermidis

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## INTRODUCTION

The prevalence of acne vulgaris sufferers in Indonesia ranges from 80-85% in adolescents with a peak incidence aged 15-18 years, 12% in women aged >25 years and 3% at the age of 35-44 years [1]. According to Solgajova [2], skin diseases will cause several psychological problems for patients, including reducing self-confidence, shame, and can even cause anxiety and fear.

Based on research by Madelina and Sulistiyaningsih [3], it was reported that the use of antibiotics for acne therapy was less effective, with many countries reporting that more than 50% of Propionibacterium acnes strains were resistant to topical macrolides. The use of antibiotics as the first choice of acne treatment therapy needs to be revisited to limit cases of antibiotic resistance. This condition encourages the development of natural antibacterial research on plants in Indonesia, namely lakum (Cayratia trifolia (L.) Domin) leaves. Propionibacterium acnes and Staphylococcus epidermidis are bacteria that can cause acne. Propionibacterium acnes is a gram-positive anaerobic bacteria that is the most dominant bacteria in acne lesions. P. acnes plays a role in the pathogenesis of acne by breaking down components of sebum, namely triglycerides into free fatty acids which are mediators that trigger inflammation [4].

Staphylococcus epidermidis is a facultative anaerobic bacterium in the form of cocci, neither spores nor motile, white or yellow bacterial colonies, and grows well at 37°C. These bacteria include normal flora on the skin and mucous membranes of humans which in certain circumstances can turn into a pathology that causes mild skin infections accompanied by abscesses [5]

The lakum plant is known to have a variety of secondary metabolites, Kumar et al., [6] reported that the ethanolic extract of lakum leaves is known to contain secondary metabolites of

flavonoids, tannins and steroids as well as carbohydrates. Lakum leaves also contain stilbensoid compounds, kaemferol, myricetin, quercetin, triterpenes, epifriedelanol [7]
Therefore, researchers are interested in conducting research that aims to determine the potential of lakum (Cayratia trifolia (L.) Domin) leaves as antibacterial on Propionibacterium acne and Staphylococcus epidermidis.

## MATERIAL AND METHODS

The tools used in this study were porcelain dishes, rotary evaporator, bunsen, ose needle, analytical balance, filter paper, glass jars, spatula, petri dish, tube, micropipette, brown paper, stirring rod, autoclave, water bath, incubator, light microscope, and other tools commonly used in the Natural Materials Laboratory, Muhammadiyah University, East Borneo.
Lakum leaves (Cayratia trifolia) were taken from Loa Ipuh Village, Tenggarong District, Kutai Kartanegara, East Kalimantan and have been determined by plants at the Laboratory of Ecology and Conservation of Tropical Forest Biodiversity, Faculty of Forestry, Mulawarman University).

**Plant material and extract preparation**
Lakum leaves (Cayratia trifolia) taken from Loa Ipuh Village, Tenggarong District, Kutai Kartanegara. A total of 1.3 kg of lakum leaves were cleaned, then dried at 40°C, then crushed. Weighed 300 grams of dried simplicia lakum leaves then put into a maceration container, then soaked with 2,250 mL of 96% ethanol solvent and left for 3-5 days while stirring occasionally. The liquid extract is evaporated using a rotary evaporator, then the thick extract is put in a glass bottle [8]

**Phytochemical screening**
Phytochemical screening was carried out on the ethanol extract of lakum leaves which included:

**Alkaloid**
Alkaloids can be identified using three reagents, namely Mayer's reagent, Bouchardat's reagent, and Dragendorf's reagent. The sample is declared positive for containing alkaloids if there are at least 2 out of 3 trials [9]
Take a sufficient sample then add 1 ml of 2N hydrochloric acid and 9 ml of distilled water, heat it on a water bath for 2 minutes, cool it and then filter it. The filtrate was experimented with the following reagents:

**Mayer's reagent**
Three drops of the filtrate, then add 2 drops of Mayer's reagent to produce a white or yellow precipitate.

**Bouchardat's reagent**
Three drops of the filtrate, then add 2 drops of Bouchardat reagent to produce a brown-black precipitate.

**Dragendorff's reagent**
Three drops of the filtrate, then add 2 drops of Dragendorff's reagent to produce a brick red precipitate.

**Flavonoid**
A number of samples were added 10 ml of hot water, boiled for 5 minutes and filtered while hot. The filtrate obtained was then taken 5 ml and then added 50 mg of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Positive flavonoids if a red, yellow, orange color is formed on the amyl alcohol layer [9]

**Tanin**
Each sample was filtered with 10 ml of distilled water, for 3 minutes then cooled and filtered. The filtrate is diluted until it is colorless, then add 1-2 drops of FeCl3 reagent if a blackish green color is formed indicating the presence of tannins [9].

**Saponin**
A number of samples were added 10 ml of hot water, boiled for 5 minutes and filtered in hot conditions. The filtrate obtained is then cooled and then shaken vigorously for 10 seconds, adding 2N HCl. Observe if foam is formed as high as 1-10 cm which does not disappear in less than 5 minutes, it indicates the presence of saponin compounds [9].

**Steroid and Triterpenoid**
A number of samples were put into a test tube, dissolved with 0.5 ml of chloroform, then added 0.5 ml of anhydrous and 3 ml of concentrated H2SO4. If a red or purple color is formed, it indicates the presence of triterpenoid compounds, while the formation of a blue or green color indicates the presence of steroids [10].

**Antibacterial activity**
All tools and materials used must be sterilized before use. Tools and materials were sterilized using an autoclave at 121°C for 30 minutes. Antibacterial test was carried out with the well diffusion method (Hole method) and Nutrient Agar (NA) as the test medium. The bacteria used in this test are bacteria Propionibacterium acne and Staphylococcus epidermidis. Lakum leaf ethanol extract was dissolved in acetone and then made into four concentrations, namely 25 g/ml, 50 g/ml, 75 g/ml and 100 g/ml. Acetone was used as a negative control because it was able to seep into the bacterial epithelium without damaging bacterial cells [11]. Then, clindamicin was used as a positive control with a concentration of 30 g/ml. Clindamycin was used as a positive control because it works as a broad-spectrum antibiotic [12]. The test was carried out with 4 times of duplication and 3 times of replication. Take 7 petri dishes to test against P. acne bacteria and 7 petri dishes to test against S. epidermidis bacteria. The liquid Nutrient Agar (NA) was poured into a petri dish as much as 20 ml, then the media was allowed to stand until it solidified. Solid media were planted with P. acne and S. epidermidis bacteria using the scatter method (spread plate). After all the plates were planted with the test bacteria, it was continued by making 4 holes in each medium using a cork borer with a hole diameter of 0.5 mm at a certain distance. Then the variance of the extract was injected as much as 20 l into the wellbore with different concentrations in each hole. After all media were given the same treatment, followed by the incubation process at 37°C for 24 hours, then

observed the clear zone formed around the hole and measured its diameter using a ruler.

**Statistical analysis**

All experiments were performed in four duplications and three replications, and the results are presented as mm (milimeter). The significance of the differences between the multiple means was determined by one-way analysis of variance (ANOVA), followed by the Tukey HSD test at a significance level of 5%.

## RESULTS AND DISCUSSION

**Extreact of lakum leaf**

The extraction results obtained a thick ethanol extract of 3.73 g with a yield value of 9.18%. Extraction in this study using the maceration method. This method was chosen because it is easy and simple. This study used 96% ethanol as a solvent. Ethanol solvent is a volatile polar compound so it is good to be used as an extract solvent [13].

**Phytochemical screening**

The results of phytochemical screening of 96% ethanol extract of lakum leaves (Cayratia trifolia L. Domin) are presented in (Table 1).

**Table I.**Results of phytochemical screening of lakum leaf extract (Cayratia trifolia L. Domin

| Compound | Reagent | | Result |
|---|---|---|---|
| | | A white | |
| | Mayer | precipitate is | (+) |
| | | formed | |
| Alkaloid | Bouchardhat | A brown-black precipitate is | (+) |
| | | formed | |
| | | A brick red | |
| | Dragendorff | precipitate is | (+) |
| | | formed | |
| | HCl, Mg, | An orange layer is | |
| Flavonoid | Amyl alcohol | formed on the amyl alcohol | (+) |
| Tannin | FeCl 1% | A blackish green color occurs | (+) |
| Saponin | HCl 2N | Foam/foam is formed | (+) |
| | Chloroform, | No turquoise ring | |
| Steroid | Anhydrous, | is formed | (-) |
| | Concentrated H2SO4 | | |

Based on the results of the screening, it was found that the ethanolic extract of Lakum leaves (Cayratia trifolia L. Domin) was positive for secondary metabolites, namely alkaloids, flavonoids, tannins and saponins, this is in line with research by Radji [14] which stated that Lakum leaves (Cayratia trifolia L. Domin) ) contains secondary metabolites including steroids, terpenoids, and tannins. Then the results of research conducted by Ilyas et al., [8] also stated that the ethanolic extract of lakum leaves contains alkaloids, flavonoids, tannins, saponins and triterpenoids. These results are also supported by research conducted by Sowmya et al.,

[15] which showed the ethanol extract of galing-galing leaves contains alkaloids, flavonoids, saponins, tannins and steroids. Alkaloids have been shown to have an antibacterial mechanism by interfering with the peptidoglycan constituent components of bacterial cells [16]. The mechanism of action of flavonoid compounds by forming complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane followed by the release of the [15]. Tannins have the ability as an antibacterial due to the ability of tannins to shrink cell walls or cell membranes, thereby disrupting the permeability of bacterial cells. While saponins have a mechanism as an antibacterial by causing leakage of proteins and enzymes from the bacterial cell [17]. Based on the phytochemical screening carried out, lakum leaves (Cayratia trifolia L. Domin) have potential as an alternative natural antibacterial. To confirm this theory, further research is needed by conducting antibacterial activity tests.

**Antibacterial activity**

The results of the antibacterial activity test against acne-causing bacteria, namely Propionibacterium acnes and Staphylococcus epidermidis are shown in (Table II). It was found that all extracts of lakum (Cayratia trifolia L. Domin) leaf had a strong inhibition zone. Antibacterial activity against

P. acne, the inhibition zone formed ranged from 14.5 – 22 mm, while for S. epidermidis the inhibition zone formed was in the range of 14 – 21.5 mm. This test uses the well diffusion epidermidis (Figure 2), it showed positive results with the formation of a clear zone around the well.

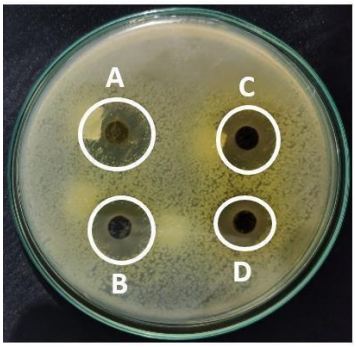


**Figure 1. Inhibition zone of ethanolic extract of lakum (Cayratia trifolia L. Domin) leaves against Propionibacterium acnes.**

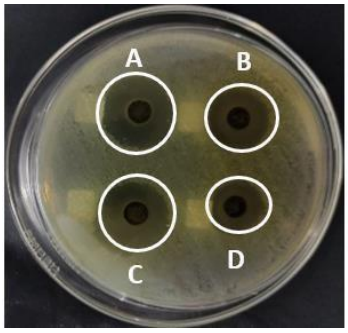


**Figure 2. Inhibition zone of ethanol extract of lakum (Cayratia trifolia L. Domin) leaves against bacteria Staphylococcus epidermidis.**

The formation of the inhibition zone in this study was caused by the content of secondary metabolites in the ethanol extract of lakum leaves (Cayratia trifolia L. Domin). In the antibacterial test against the bacteria Propionibacterium acnes, the greatest antibacterial activity was obtained at a concentration of 100% which was 20.5 mm as well as for Staphylococcus epidermidis bacteria at 21.5 mm. This is in line with Lovista's [19] statement that the greater the concentration of the extract, the higher the inhibition against bacteria. The results of the antibacterial activity test in this study are also supported by research Sari et al., [20] stated that the ethanolic extract of the leaves of Lakum (Cayratia trifolia
L. Domin) has antimicrobial activity against Candida albicans and Staphylococcus aureus. In addition, research conducted by Sari et al., [20] also stated that the ethanolic extract of lakum leaves (Cayratia trifolia L. Domin) has antibacterial activity against Staphylococcus aureus.
The results of this test showed that the greatest antibacterial activity was found at a concentration of 100%, but the inhibition zone formed in P. acnes and S. epidermidis was still smaller when compared to the positive control using clindamycin with an average diameter of 39.5 mm in P. acnes bacteria and 43.5 mm in bacteria S. epidermidis. The magnitude of the inhibitory power on the positive control because clindamycin is an antibiotic that works by inhibiting protein synthesis is also the reason for using clindamycin as a positive control in this study [12]. method because in general the antibacterial activity using this method has better results than the antibacterial activity using the disc method. This is presumably due to the osmosis process of the extract concentration that occurs homogeneously and efficiently so that it is more effective in inhibiting bacterial growth [18]. From the results of observations on the inhibition of the ethanolic extract oflakum (Cayratia trifolia L. Domin) leaves againstPropionibacterium acnes (Figure 1) and Staphylococcus

**Table II.** The results of the observation of the inhibition of lakum leaves (Cayratia trifolia L. Domin) Strain Compound Inhibition zone diameter (mm)

| | | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|---|
| P. acnes | K(+) | 39,5 | 39,5 | 39,5 | 39,5 | 39,5 | 39,5 | 39,5 |
| | K(-) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100% | 19,5 | 18,5 | 22 | 18,5 | 20,5 | 20,5 | 20,5 |
| | 75% | 19,5 | 19,5 | 21,5 | 16,5 | 19 | 18,5 | 19,5 |
| | 50% | 16,5 | 19,5 | 19,5 | 16,5 | 16,5 | 18 | 18 |
| | 2 5% | 21,5 | 14,5 | 16 | 15,5 | 14,5 | 15,5 | 15 |
| | K(+) | 43,5 | 43,5 | 43,5 | 43,5 | 43,5 | 43,5 | 43,5 |
| K(-) | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. epidermidis* | 100% | 20,5 | 20,5 | 20,5 | 20,5 | 21,5 | 21,5 | 21,5 |
| | 75% | 18 | 22 | 18 | 19,5 | 19 | 19,5 | 19,5 |
| | 50% | 15 | 19 | 15 | 18,5 | 20,5 | 19,5 | 21 |
| | 25% | 18 | 14 | 16,5 | 14,5 | 18,5 | 17,5 | 19,5 |

The data obtained in this study in the form of the diameter of the inhibition zone were analyzed by One-way Anova. Prior to the One-way Anova test, the data were tested using the Saphiro-Wilk test and Levene's test ($p > 0.05$). Obtained data that is normally distributed and has a homogeneous variance. One-way Anova analysis on the diameter of the inhibition zone showed that there was a significant effect on the growth of acne-causing bacteria, the probability value (p) = 0.000 or
(p) = < 0.05. Thus, it can be stated that the ethanol extract of lakum leaves (Cayratia trifolia L. Domin) has antibacterial power against the growth of bacteria that cause acne. The results of the analysis were continued with the Tukey HSD (Honest Significant Different) test to find out which treatment groups had a significantly different effect. The results of the Tukey HSD test for the group of ethanol extract of lakum leaves (Cayratia trifolia L. Domin) with concentrations of 25%, 50%, 75% and 100%, negative control and positive control had significant differences.

## CONCLUSION

Lakum leaf (Cayratia trifolia L. Domin) ethanol extract has antibacterial activity against acne-causing bacteria. The greatest inhibition was obtained at 100% extract concentration with an average diameter of 20.5 mm on Propionibacterium acnes and 21.5 mm on Staphylococcus epidermidis bacteria. The ethanol extract of positive lakum leaves contains alkaloids, flavonoids, tannins and saponins which are also compounds that have antibacterial activity.

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**Ethical Approval**

This study did not use experimental animals so it does not require approval from the ethics committee.

**Conflict Of Interest**

The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Authorship**

Lutfi Chabib : main idea of research, data analysis
H. Hamzah : final approval, data analysis
I. Luthfiyah : plant determination and extraction, antibacteri testing, draft writing

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