



Antibacterial Activity Test of *Passiflora Foetida* Linn Leaves Extract on *Propionibacterium Acne*

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Abstract

Acne is a chronic inflammatory skin disease affecting the sebaceous glands, primarily caused by the growth of *Propionibacterium acne*. A significant natural antibacterial agent against the bacteria is *Rambusa* (*Passiflora foetida* Linn) leaves, which contain active compounds such as alkaloids, flavonoids, tannins, saponins, and terpenoids/steroids. Therefore, this research aimed to evaluate antibacterial activity of ethanol extract from *Rambusa* leaves against *Propionibacterium acne* growth and determine the effective concentration of test solution inhibiting bacteria growth. The leaves were subjected to extraction through the maceration method using 96% ethanol solvent. The resulting extract was tested for antibacterial activity using the disc diffusion method. This research used three concentrations, including 10% b/v, 15% b/v, and 20% b/v. The positive control used 2 µg clindamycin disks, and the negative control used aqua pro injection. The analysis results showed that there was no clear zone area around disks containing *Rambusa* leaves extract at any concentration. In conclusion, the ethanol extract of leaves lacked antibacterial activity against *Propionibacterium acne*.

Introduction

Acne, a chronic inflammatory skin disease affecting the sebaceous glands (Lestari et al., 2020), is characterized by two types, including non-inflammatory (whiteheads and blackheads) and inflammatory (pustules, papules, or nodules) within the pilosebaceous unit (Elvira, 2019). Approximately 85% of individuals experience acne, predominantly during the stage of adolescence. The highest occurrence is observed in females aged 14 - 17 (83-85%) and males aged 16 - 19 (95-100%) (Saragih et al., 2016). Although not life-threatening, acne carries significant medical and psychological consequences due to its potential to damage appearance and leave marks on facial skin, resulting in stress, worry, despair, and reduced self-confidence levels among adolescents (Syahputra et al., 2021).

Propionibacterium acne is a key bacterium triggering acne, as evidenced by Pangestu et al. (2017). Despite the widespread use of chemical drugs such as erythromycin, clindamycin, tetracycline, and benzoyl peroxide to treat acne, their excessive misuse can lead to bacterial resistance, posing potential adverse effects. Therefore, there is a growing need to explore natural antibacterial compounds devoid of adverse effects, including using natural ingredients from the environment (Marselia et al., 2015).

According to (Mulyani et al., 2022), *Rambusa* leaves (*Passiflora foetida* Linn) are known for their antibacterial activity. This plant is commonly found in various regions, recognized as a wild plant inhabiting bush and highlands. Previous investigations (Benita, 2023) showed the

presence of chemical compounds in Rambusa leaves extract, including alkaloids, flavonoids, saponins, tannins, and terpenoids. The plant has been acknowledged for its medicinal benefits, being used as herbal medicine to treat a variety of conditions such as urinary tract infections, anxiety, headaches, nervousness, sleeplessness, skin diseases, diarrhea, throat and ear infections, asthma, and itching (Foudah et al., 2019). Further research by Sari & Puspitasari (2021) found that Rambusa leaves extract contains flavonoid and alkaloid compounds with antimicrobial activity against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Additionally, ethanol extract from the plant has shown antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria (Noviyanti et al., 2014).

Based on the description above, the phytochemical content in Rambusa leaves presents an opportunity for analysis as an ingredient to inhibit the growth of *Propionibacterium acne*. There have been no investigations regarding Rambusa leaves as an antibacterial against *Propionibacterium acne*. Therefore, this research aims to analyze antibacterial activity of Rambusa leaves extract against *Propionibacterium acne*.

Methods

Type of Research

This research adopted experimental methods and was conducted in the Natural Materials Laboratory and Microbiology Laboratory, Pharmacy Study Program, Faculty of Applied Science, Muhammadiyah Sorong University of Education, from February to April 2024. The procedural stages included sample selection and collection of Rambusa leaves, simplisia preparation, extract preparation, phytochemical screening, sterilization of tools and test solutions, preparation of nutrient agar (NA) media, suspensions of *Propionibacterium acne*, testing antibacterial activity, and data analysis.

The research was conducted between February and April 2024 at the Natural Materials Laboratory and Microbiology Laboratory, Pharmacy Study Program, Faculty of Applied Science, Muhammadiyah Sorong University of Education. The population included Rambusa plants obtained from Osok Street, Aimas, Sorong, and Southwest Papua, Indonesia. The sample consisted of Rambusa leaves which had the criteria of being green, fresh, and undamaged.

Research instruments

The equipment used included an autoclave, aluminum foil, sieve, blender, Bunsen burner, stirring rod, glass bottle, petri dish, funnel, Erlenmeyer flask (Pyrex), beaker (Pyrex), measuring cup, hand scoop, hot plate, incubator, ose needle, vernier caliper, cotton, label paper, filter paper, disc paper, laminar air flow, micropipette, dropper, tweezers, plastic wrap, test tube rack, marker, mattress rope, analytical balance, test tube, jar, and water bath.

The materials used consisted of clindamycin antibiotic disks (2 µg), aqua pro injection, sterile distilled water, hydrochloric acid (HCl 2N), *Propionibacterium acne*, barium chloride (BaCl₂ 1%), iron (III) chloride (FeCl₃), Rambusa leaves, ethanol (96%), sulfuric acid (H₂SO₄ 1%), NA media, sodium chloride (NaCl 0.9%), Pb II acetate reagent, Mayer reagent, Bouchardat reagent, and liberman-burchard reagent.

Working procedure

Samples of Rambusa leaves weighing 5 kg were collected and subjected to wet sorting. They were thoroughly washed under running water, drained, knitted, and dried using an oven at 40°C. Subsequently, the dried samples were pulverized with a blender and sieved to obtain a fine and homogeneous powder. The resulting powder was further weighed and placed into a closed container (Gerung et al., 2021).

To extract compounds from Rambusa leaves, 500 g of the simplisia was soaked in 2000 mL of 96% ethanol solvent in a 1:4 ratio until fully submerged. The maceration container was sealed

and left for 72 hours, with occasional stirring and protection from direct sunlight. The mixture was then filtered using filter paper to separate the filtrate from the residue. To enhance compound extraction, remaceration was performed with 1500 mL of 96% ethanol extract in a 1:3 ratio for 48 hours. The filtrate from both maceration and remaceration was combined and evaporated using a water bath at 40°C until a thick extract was obtained (Tamimi et al., 2020).

Phytochemical screening

A 2 mL sample was placed in test tube, and 2 mL of 2% HCl was added, heated for 5 minutes, cooled, and filtered. The filtrate was subjected to testing using Mayer and Bouchardat reagents (2-3 drops). Alkaloid compounds were identified by the formation of white or yellow and dark brown precipitates (Ningsih, 2017). Approximately 2 mL sample was placed in test tube, and 2-3 drops of Pb II acetate were added. The presence of flavonoid compounds was characterized by the formation of a yellow precipitate (Cahyaningsih et al., 2017).

A thick extract of 2 mL was mixed with FeCl₃ reagent, and the formation of a dark blue or blackish-green color showed the presence of tannin compounds (Ningsih, 2017). About 2 mL thick extract was mixed with 10 mL hot water in test tube, cooled, and shaken until foam appeared. After the solution was allowed to stand for 10 minutes, one drop of HCL 2N was added. The constant froth showed the presence of saponin compounds (Ningsih, 2017).

A 2 mL sample was mixed with an anhydrous acetic acid reagent (2 drops) and concentrated sulfuric acid (1 drop). The presence of steroid/terpenoid compounds was showed by the formation of blue or green rings and red or purple coloration (Nurjannah et al., 2022). Before commencing work, tools and materials were sterilized using an autoclave at 121°C for 15 minutes to eliminate all types of living organisms such as protozoa, fungi, bacteria, mycoplasma, or viruses (Charisma, 2020).

Preparation of NA media

A total of 7 g of NA media was dissolved into 250 mL of sterile distilled water using an Erlenmeyer flask. It was homogenized on a hot plate until boiling, covered with aluminum foil, and sterilized in an autoclave at 121° C for 15 minutes (Usman, 2020).

Preparation of test bacteria

Propionibacterium acne was obtained from pure culture. A single ose of bacteria was inoculated onto a tilted NA medium and incubated at 37°C for 24 hours (Usman, 2020). After rejuvenation for 24 hours, a sterile ose needle was used to collect the bacteria. They were then inoculated into test tube containing 10 mL of 0.9% NaCl solution and homogenized until the turbidity matched that of the Mc. Farland standard (3×10^8 CFU/ml) (Usman, 2020).

Antibacterial activity test

About 15-20 mL of NA medium was poured into a petri dish and allowed to solidify. A single ose of bacteria, measured based on McFarland standards, was then inoculated evenly on the surface of the solidified medium using a sterile cotton swab in a zigzag manner. After a few minutes, the suspension was absorbed into the agar medium.

Sterile disks were then aseptically transferred using sterile tweezers into the previously prepared extract solution with concentrations of 10%, 15%, and 20%, and left for 15 minutes until saturated. Subsequently, the soaked disks were aseptically transferred to NA medium containing *Propionibacterium acne*, starting with the positive control (clindamycin disk), followed by the negative control (aqua pro injection), and the disks containing various concentrations of ethanol extract solution of Rambusa leaves. These disks were placed into the same petri dish, maintaining a distance of 1-2 cm between each disk along the edge of the dish. The treated petri dish was then incubated for 24 hours at 37°C, and this process was replicated

three times. Antibacterial activity test treatment was carried out aseptically in a laminar airflow (Putri, 2021).

Observation and measurement

The diameter of the inhibition zone (clear zone) formed around the disk after 24 hours was observed and measured using a caliper. The calculation was performed using the following formula (Oroh et al., 2015).

$$\text{Zone of inhibition} = \frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Result and Discussion

Table 1. Rendement of Rambusa Leaves Extract (*Passiflora foetida* Linn.)

Simplisia	Sample weight	Powder weight	Extract weight	Rendement
Rambusa Leaves	5 kg	500 g	88 g	17.6%

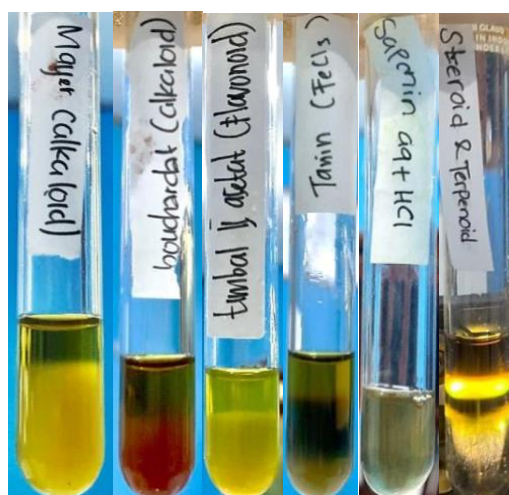


Figure 1. Phytochemical screening results of Rambusa leaves

Table 2. Phytochemical Screening Results of Rambusa Leaves

Group of compounds	Reagents	Observation Result	Test Result
Alkaloid	Mayer	White or yellow precipitate.	+
	Bouchardat	Dark brown precipitate.	+
Flavonoid	Pb II acetate	Yellow precipitate.	+
Tannin	FeCl ₃	Dark blue or blackish green color.	+
Saponin	HCl 2N	Stable froth.	-
Terpenoid/steroid	Liberman-burdchard	Blue or green color ring and red. or purple color.	+

Description: (+) Contains secondary metabolite compounds

(-) Does not contain secondary metabolite compounds

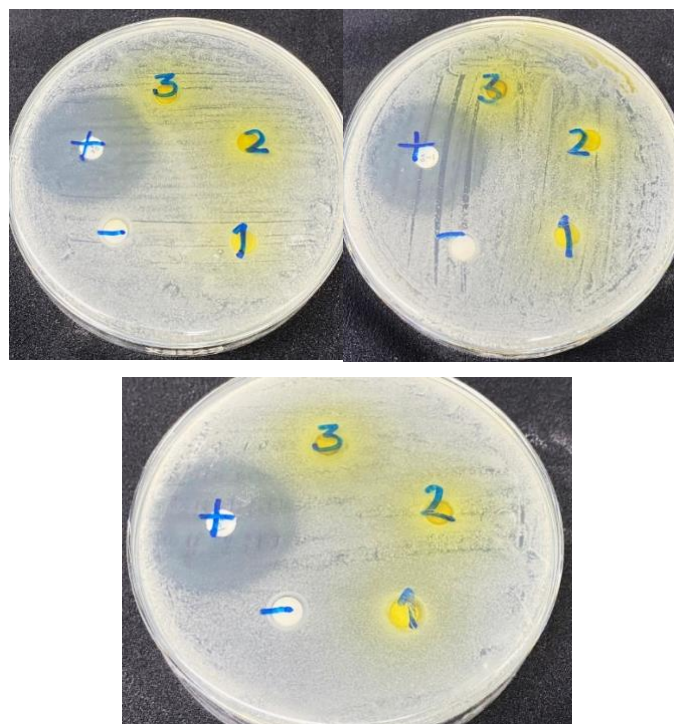


Figure 2. Antibacterial activity test results of Rambusa leaves extract against *Propionibacterium acne*

Description:

- 1 = 10% concentration
- 2 = 15% concentration
- 3 = 20% concentration
- + = Positive control (Clindamycin disk 2 microgram)
- = negative control (aqua pro injection)

Table 3. Results of Antibacterial Activity Test of Rambusa Leaves Extract

Concentration (%)	Inhibition zone diameter (mm) of <i>Propionibacterium acne</i>			Average (mm)
	Replication 1	Replication 2	Replication 3	
5 %	0 mm	0 mm	0 mm	0 mm
10 %	0 mm	0 mm	0 mm	0 mm
20 %	0 mm	0 mm	0 mm	0 mm
Negative control (aqua pro injection)	0 mm	0 mm	0 mm	0 mm
Positive control (clindamycin disk 2 µg)	28 mm	28 mm	27 mm	27.66 mm

Description: 0 = no zone of inhibition

The test extract used was derived from Rambusa leaves, which were macerated using 96% ethanol as the solvent. The maceration method was useful for extracting compounds that were not heat resistant, and the tools used were relatively simple, cheap, and easy to obtain. The principle of maceration was that the solvent had the ability to penetrate the plant cell membrane, dissolving the cell contents due to the differences in concentration between the solution inside and outside the cell. The results, as presented in Table 1, showed that 500 g of powdered leaves yielded 88 g of viscous extract. This corresponded to an extraction yield of 17.6%, meeting the requirements of the Indonesian Herbal Pharmacopoeia, which stipulated a minimum yield of 10%. A higher yield showed the extract produced was greater and that the extraction method used was more effective (Badriyah & Farihah, 2023). These results were closely in line with

previous research conducted by (Sari & Puspitasari, 2021), which reported a yield of 14.5% using the same extraction method and solvent.

The phytochemical screening test presented in Table 2 showed the presence of secondary metabolite compounds in Rambusa leaves extract, including alkaloids, flavonoids, tannins, and terpenoids /steroids, evidenced by deposition and color changes. However, there were differences in the types of identified compounds compared to previous investigations conducted by (Benita, 2023), particularly in the saponin group. The saponin test on Rambusa leaves extract showed negative results, as the foam produced dissipated quickly.

The differences in secondary metabolite compounds could be attributed to environmental conditions, plant type (varieties), physiological state (old or young), and chemical properties. Environmental factors such as temperature, humidity, solar radiation, wind, water availability, and light during photosynthesis significantly influence plant physiology, morphology, and life cycle. Plants typically adapt to these environmental changes, which could affect the production of secondary metabolite compounds in Rambusa leaves. For instance, temperatures that were extremely high or low could disrupt enzyme activity and metabolic processes in plants. This inhibited the production of secondary metabolites because the biosynthesis pathway was disrupted. Plants needed light for photosynthesis and energy production, and insufficient or low light could inhibit the photosynthesis process. Furthermore, inappropriate soil pH, poor soil texture, or soil contamination had the tendency to interfere with nutrient absorption, inhibiting the production of secondary metabolites. Plants might produce different secondary metabolite compounds at various growth phases or in response to biotic or abiotic stress, typically adapting to natural changes. These factors influenced the secondary metabolite compounds produced by Rambusa leaves (Narulita, 2017).

Table 3 showed that 96% ethanol extract of Rambusa leaves at varying concentrations did not have antibacterial activity against *Propionibacterium acne*. This was evident from the absence of an inhibition zone around the disks containing extract. Even after three repetitions, there was still no clear inhibition zone around the disks. As a negative control, aqua pro injection failed to inhibit *Propionibacterium acne* growth, while 2 µg clindamycin disk as a positive control provided inhibition with an average zone diameter of 27.66 mm.

The results differed from previous reviews, where (Wijayanti et al., 2022), (Mulyani et al., 2022), and (Safrida et al., 2023) reported that Rambusa leaves contained antibacterial compounds, showing weak to strong inhibition zones. Several factors might accounted for this disparity in antibacterial activity testing of the 96% ethanol extract of Rambusa leaves against *Propionibacterium acne*. For instance, variations in the quality of raw materials could significantly influence test results of antibacterial activity. Rambusa leaves grown in different locations could vary in composition, as evidenced by (Suryati et al., 2018). In this research, leaves were obtained from Osok Street, Aimas, Sorong Regency, an area with distinct climate, temperature, and soil conditions. The dry soil where the sample was collected might have influenced extraction process, resulting in lower quality and quantity of extract compared to leaves grown in more fertile soil with a higher water content (Putri, 2022)

The concentration of antibacterial compounds could affect test results of antibacterial activity. A higher level of active ingredients was usually correlated with greater inhibition of bacterial growth. In this research, concentrations of 10%, 15%, and 20% were used, based on previous investigations conducted by (Wijayanti et al., 2022) and (Mulyani et al., 2022). While the investigations reported weak to strong inhibition zones, the current analysis found no significant antibacterial activity at any concentrations (Apriyuslim, 2015). The method used to test the secondary metabolite compounds in this research was qualitative, specifically the tube test, which only detected the presence or absence of the compounds, thereby precluding quantification of their levels within Rambusa leaves.

Laboratory contamination might affect the research results, potentially reducing antibacterial activity of the 96% ethanol extract. Factors such as room conditions, tool sterilization procedures, and ancillary equipment maintenance decreased extract quality, thereby affecting the expected antibacterial activity. Environmental conditions including room ventilation, air circulation, occupancy, and practitioner hygiene standards could introduce bacteria contamination (Bidin, 2017).

Conclusion

In conclusion, this research showed that the 96% ethanol extract of Rambusa leaves lacked antibacterial activity against the growth of *Propionibacterium acne*. Further investigations were recommended to carry out antibacterial activity test using alternative components of Rambusa plant such as fruit peel, stems, and seeds. Additionally, quantitative phytochemical analysis of the 96% ethanol extract through spectroscopic methods and compound isolation procedures was advised to identify antibacterial compounds targeting *Propionibacterium acne*.

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