

# Antibacterial activity of methanolic extract of *Acrostichum Aureum Linn* leaves against *Propionibacterium acnes*

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**Abstract.** *Propionibacterium acnes* is the main organism that leads to acne. *P. acnes* belongs to a group of gram-positive bacteria. Antibiotics can be used to relieve acne, but they could lead bacteria to accumulate resistance. As a result, a safe and cost-effective treatment strategy is required. An instance is using *Acrostichum aureum Linn* leaves, which contain several bioactive chemicals that function as antibacterial. This study aims to identify the bioactive compounds and antibacterial activity of the methanolic extract of *Acrostichum aureum Linn* against *Propionibacterium acnes*. The study started with extraction (maceration method) with methanol solvent for 3 days, followed by phytochemical tests to identify bioactive compounds, and an antibacterial activity test using the disc diffusion method. Phytochemical tests on the methanol extract of *Acrostichum aureum Linn* leaves revealed five bioactive compounds consisted of alkaloids, flavonoids, tannins, steroids, and saponins. Antibacterial tests against *Propionibacterium acnes* exhibited no inhibition zone at concentrations of extract from 5% to 75%. However, at 100% concentration, indicating strong inhibition of *P. acnes* growth (diameter zone is 16 mm). Based on the research, it is conceivable to conclude that the extract has strong antibacterial activity against *Propionibacterium acnes* at a concentration of 100%.

## 1 Introduction

*Propionibacterium acnes*, Gram-positive, anaerobic bacterium prevalent on the skin of people, is associated with acne vulgaris. *Acne vulgaris* is one of the most prevalent dermatological conditions worldwide, particularly affecting adolescents and young adults [1]. The pathogenesis of acne involves multiple factors, including hyperkeratinisations, excessive sebum production, hormonal imbalances, and bacterial colonization, particularly by *Propionibacterium acnes*. *P. acnes* plays crucial part to activates the innate immune response to cause inflammation, which leads to, pustules, papules, comedowns, and in extreme cases, cysts, and nodules. Although antibiotics like clindamycin, erythromycin, and

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tetracycline are widely used to control bacterial proliferation and inflammation, the rise of antibiotic resistance has emerged as a significant challenge in acne management [2-5].

In light of the growing concern over antibiotic-resistant strains of *P. acnes*, there is increasing interest in identifying new therapeutic agents, particularly from natural sources. For centuries, medicinal plants have been used in traditional medicine to treat skin conditions, and recent scientific research has explored their potential as effective and safer alternatives to synthetic antibiotics. One such plant is *Acrostichum aureum* Linn, a species of mangrove fern commonly found in coastal and estuarine ecosystems across tropical and subtropical regions. *Acrostichum aureum* is a tropical plant found in wetlands and mangroves, traditionally used to treat a variety of diseases and ailments [6,7]. This plant exhibits various pharmacological properties, supported by many secondary metabolites such as phthalates, flavonoids, sterols, and terpenoids [8]. In various traditional medicinal systems, particularly in Southeast Asia, *A. aureum* has been utilized to treat ailments such as fever, diarrhea, and wounds due to its purported anti-inflammatory, antioxidant, and antimicrobial properties [9, 10].

Previous studies on *A. aureum* have reported bioactive compounds, such as flavonoids, tannins, sterol, terpenoid, glycosides, alkaloid, saponins, and phenolic acids, which are known to exhibit anticancer, antiulcer, anti-tumor, antioxidant, antibacterial and anti-inflammatory effects [11]. However, despite its promising pharmacological potential, limited research has been conducted to specifically evaluate the antibacterial efficacy of the methanolic extract of *A. aureum* leaves against *P. acnes*. An in vitro investigation is essential to assess the antibacterial abilities of the extract, which includes bioactive compounds. Therefore, the aim of this study is to screening secondary metabolites and evaluate the inhibitory effect of *A. aureum* Linn extract against *P. acnes*.

## 2 Research methods

### 2.1 Times and place

The study was conducted between January and March 2024. Leaves of *Acrostichum aureum* were collected in Cotlamkuweuh village, located in the Meuraxa District of Banda Aceh. Extraction activities and phytochemical tests were carried out at the Marine Chemistry and Biotechnology Laboratory, Faculty of Marine and Fisheries, Syiah Kuala University, while antibacterial tests were performed at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

### 2.2 Tools and materials

The tools used are micropipette, analytical balance, rotary evaporator, glassware UV-Vis spectrophotometer, incubator, hot plate, autoclave, test tube, light microscope, stirring rod, pipettes, loop needle, refrigerator, calliper, hot place, petri dishes, Bunsen lamps, and aluminium foil. The materials used *Acrostichum aureum* Linn, bacterial safety cabinet, methanol, chloride acid, sulfuric acid, potassium iodide, aquadest, dimethyl sulfoxides, clindamycin, nutritional agar, muller hilton agar, cotton swabs, and bacterial tests for *P. acnes*.

### 2.3 Sample extraction

A sample-to-solvent ratio of 1:5 was employed to macerate 100 grams of dried *Acrostichum aureum* Linn leaves in methanol for 72 hours. The extraction process was carried out using

filter paper for separation, followed by condensation with a rotary evaporator at 40°C to yield a viscous extract. This extract was subsequently evaluated through phytochemical analyses

## **2.4 Phytochemical test**

Secondary metabolites, including saponins, alkaloids, steroids, tannins, phenolics, terpenoids, and flavonoids, were analyzed in the phytochemical screening of *Acrostichum aureum* Linn extracts. The screening was performed using standardized methodologies [12].

## **2.5 Antibacterial activity**

### **2.5.1. Preparation agar media**

In an Erlenmeyer flask, combine 0.56 grams of powdered nutrient agar (NA) with 150 mL of H<sub>2</sub>O. Heat the mixture to 40°C while stirring on a hot plate until completely dissolved. The flask should then be covered with plastic wrap and sterilized in an autoclave at 121°C for 15 minutes.

### **2.5.2. Bacteria regeneration**

*P. acnes* isolates were introduced at a concentration of 1 inoculating loop into 25 mL of Mueller-Hinton Agar (MHA) and cultivated for six hours in an incubator shaker at 28°C. The antibacterial analysis necessitates the use of fresh bacterial colonies.

### **2.5.3 Antibacterial activity test**

In this study, the disc diffusion method was employed to evaluate the effectiveness of the antibiotics. Extracts of *Acrostichum aureum* Linn were prepared at concentrations of 5%, 25%, 50%, 75%, and 100%. The antibacterial activity of the paper discs was assessed by soaking them in 20 µL of the crude extract of *Acrostichum aureum* and allowing them to air dry. The paper discs were then placed on top of nutrient agar (NA) that had been inoculated with the test bacteria. In this case, *Propionibacterium acnes* (a gram-positive bacterium) was used, and the mixture was incubated at 37°C for one day. A calliper was used to measure the diameter of the clear zone created around the discs

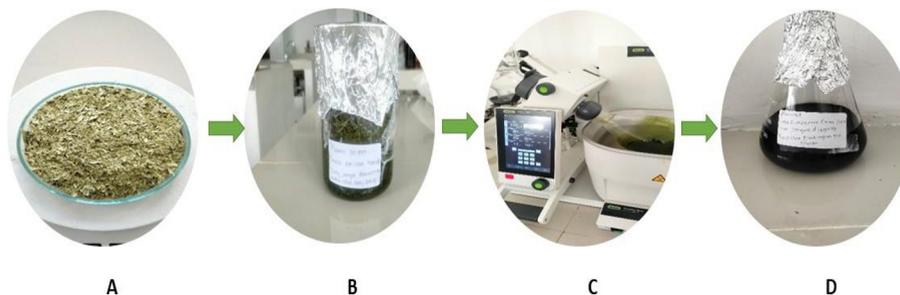
## **3 Result and discussion**

### **3.1 Extraction of *Acrostichum aureum* Linn**

Extraction is the initial stage in separating the desired natural products from their base components. The extraction using maceration techniques is simple, inexpensive, and highly beneficial for isolating bio compounds. By immersing the sample in an organic solvent, the cell walls and membranes in the cytoplasm break down, allowing the active substances to dissolve. This results in a more complete extraction, especially since the immersion duration can be adjusted for optimal results. The choice of solvent in the maceration process plays a crucial role in its effectiveness, depending on the solubility of the natural compounds in the solvent. Methanol is commonly used in this isolation process because it can dissolve a wide range of secondary metabolites [13, 14].

*Acrostichum aureum* Linn leaves were cleaned with running water to remove dirt and dust affixed to them prior to extraction. This step is crucial for enhancing the accuracy of the

chemical content identification of *Acrostichum aureum* leaves. The cleansed leaves were then air-dried for a week, outside of direct sunlight. Afterward, the sample was sliced into small fragments and mashed until smooth, resulting in a fine powder. The process of extraction is shown in Figure 1.



**Fig. 1.** Extraction process of *Acrostichum aureum* Linn leaves.

Fig 1 represents the maceration method for extracting *Acrostichum aureum* Linn leaves using methanol solvents. Figure A shows fine powder of dried *Acrostichum aureum* Linn leaves. Figure B shows dry powder are immersing with methanol for 3 days. After that, residue is separated with filter paper and the extract is concentrated by vacuum evaporator with rotary evaporator, as shown in figure C and the crude extract obtained is as shown in Fig 1D.

### 3.2 Phytochemical screening

Phytochemicals are naturally occurring chemical compounds in plants that contribute to health benefits. Phytochemical screening is a process used to identify and analyse the bioactive compound present in plants that has biological activities including antioxidant, antimicrobial, antibacterial, anti-inflammatory, and anticancer properties [15]. According [16,17] the phytochemical components of plants are key factors in determining their medicinal properties. Important phytochemicals, including alkaloids, flavonoids, terpenes, phenolics, glycosides, steroids, saponins, and tannins, are widely distributed throughout these plants. The phytochemical test identified steroid, tannins, flavonoid, saponins, and alkaloids in the methanol extract of *Acrostichum aureum* Linn. Below is Table 1 showing the test results.

**Table 1.** Phytochemical of *Acrostichum aureum* Linn extract.

Secondary Metabolites	Methods	Results
Alkaloids	Dragondroff	+
Flavonoids	Shinoda	+
Tannin	Braymer	+
Saponins	Froathing	+
Steroid	Liebermann burchad	+

The results of the phytochemical tests indicated that the raw extract of *Acrostichum aureum* Linn contained alkaloids and saponins. The pharmacological effects of alkaloids and saponins on bacteria have been reported by [18, 19].

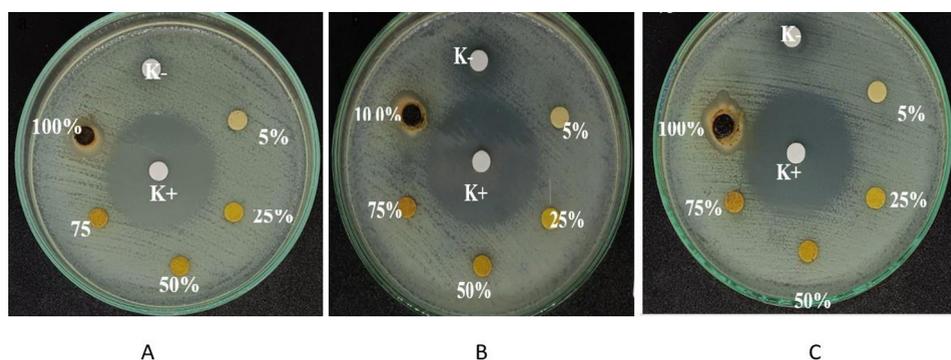
### 3.3 Antibacterial test

The study investigated the antibacterial activity of the methanolic extract of *Acrostichum aureum* Linn leaves against *P. acnes*, a bacterium implicated in acne development. The results of the antibacterial activity test of the methanolic extract of *Acrostichum aureum* Linn against *P. acnes* bacteria after 24 hours of treatment at concentrations of 5%, 25%, 50%, 75%, 100%, as well as with negative and positive controls, are shown in Table 2.

**Table 2.** Antibacterial activity test results against *P. acnes*

Concentration (%)	Inhibition Zone (mm)			Average (mm)	Inhibition criteria
	I	II	III		
5%	0	0	0	0	-
25%	0	0	0	0	-
50%	0	0	0	0	-
75%	0	0	0	0	-
100%	15	16	17	16±0,81	Strong
Clindamycine (+)	20	24	19,5	21,17±2,82	Very Strong
Methanol (-)	0	0	0	0	-

Based on the data in Table 2, *Acrostichum aureum* Linn leaf extract shows antibacterial activity. At extract concentrations ranging from 5% to 75%, the extract was unable to inhibit the growth of *P. acnes* bacteria. This is because the concentration of active compounds in *Acrostichum aureum* Linn was not strong enough to denature the bacterial cell wall. However, at a concentration of 100%, an inhibition zone began to form, categorized as strong, with the size of the inhibition zone increasing as the extract concentration increased. This indicates that the *Acrostichum aureum* Linn leaves, at a concentration of 100%, have a better ability to inhibit the growth of *P. acnes* bacteria [20]. Clindamycin, used as a positive control, formed a very strong inhibition zone. According [21], the concentration of the extract affects the diffusion rate of the active substance; the higher the concentration of the extract, the greater the antibacterial activity and the larger the diameter of the inhibition zone formed [22]. According [23, 24] the higher the concentration, the more active compounds are present, which increases the effectiveness in inhibiting bacteria and results in a larger inhibition zone.



**Fig. 2.** Inhibition zone of *acrostichum aureum linn* extract.

*Acrostichum aureum* Linn extract can suppress the spread of *P. acnes* bacteria is attributed to the presence of bioactive compounds in the plant, such as alkaloids, flavonoids, tannins, and saponins. Alkaloid substances work as antibacterial agents by destroying the peptidoglycan components of bacterial cells, inhibiting the breakdown of a continuous cell wall, and inducing cell death. Additionally, alkaloids inhibit protein synthesis, thereby interfering with bacterial metabolism [25]. Flavonoids work by inhibiting the formation of complex bonds in the bacterial cell membrane, damaging the cell membrane. Tannin compounds cause bacterial cells to lyse by disrupting cell wall synthesis, leading to imperfect cell wall formation and bacterial death. They also inactivate enzymes and interfere with bacterial protein transport. The antibacterial mechanism of saponins involves causing protein leakage; their active substances act similarly to detergents, lowering the surface tension of bacterial cell walls and damaging their permeability [26].

## 4 Conclusion

The research findings reveal five of bioactive compounds contained in the extract of *Acrostichum aureum* Linn leaves like tannin, steroid, flavonoids, alkaloids, and saponins. The methanolic extract of *Acrostichum aureum* Linn revealed antibacterial effect on *P. acnes*, not inhibiting at concentrations of 5% to 75%, and strongly inhibiting it at a concentration of 100%. Comprehensive clinical studies are needed to confirm the results and ensure the extract's medical application.

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