ANTIBACTERIAL ACTIVITY TEST OF LAMTORO (LEUCAENA LEUCOCEPHALA L.) SEED INFUSION AGAINST PROPIONIBACTERIUM ACNES

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Abstract

Background: Lamtoro, also known as Chinese petai, is a wild plantaa found throughout Indonesia, including Papua. Lamtoro plants are commonly used by people as traditional medicine to heal wounds, treat worms, and treat acne. The part used is the seeds of the lamtoro plant (*Leucaena leucocephala* L.), which contain alkaloids, flavonoids, saponins, and tannins, which have the potential as antibacterials. **Objective:** to determine the inhibitory activity of lamtoro seed infusion extract (*Leucaena leucocephala* L.) at concentrations of 12.5%, 25%, and 50% against *Propionibacterium acnes*. **Method:** Extraction was performed using the infusion method, and antibacterial activity was tested using the agar diffusion technique.**Results:** The three concentrations tested had inhibitory power against *Propionibacterium acnes* with an average diameter formed at a concentration of 12.5% of 8.19 mm, a concentration of 25% of 9.60 mm, and a concentration of 50% of 9.76 mm. **Conclusion:** In this study, it can be concluded that the extract of lamtoro seed infusion with concentrations of 12.5%, 25% and 50% has moderate antibacterial activity against *Propionibacterium acnes*.

Keywords: Antibacterial, infusion, *Leucaena leucocephala* L, *Propionibacterium acnes*

1. INTRODUCTION

Acne is a skin problem that often occurs during adolescence and into adulthood. It is characterized by the appearance of blackheads, pustules, papules, cysts, and nodules on the neck, face, upper arms, back, and chest (Winato *et al*., 2019).

Common bacteria that infect acne are *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. *P. acnes* is the primary target of antibacterial treatments for acne. *P. acnes* is a slow-growing, Gram-positive anaerobic bacterium often found in acne. *P. acnes* plays a role in the pathogenesis of acne vulgaris by producing lipases that break down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when interacting with the immune system and contribute to the development of acne vulgaris (Winato *et al*., 2019).

The use of natural ingredients as traditional medicine in Indonesia has recently increased, with some even being manufactured on a large scale. Traditional medicine is considered to have fewer side effects than chemical-based medications. Therefore, alternative acne treatments utilizing natural ingredients are needed. One of the medicinal plants often used as a source of medicine is the lamtoro plant, also known as petai cina (*Leucaena leucocephal*a L.), a wild plant found almost throughout Indonesia. Leucaena seeds contain secondary metabolites such as alkaloids, flavonoids, saponins, and

tannins, which have antibacterial potential. This plant has also been reported to have antibacterial, antidiabetic, anti-inflammatory, anticancer, anthelmintic, and antioxidant properties (Lailis *et al*., 2024).

To obtain the active ingredient in seeds, the infusion method was used. Infusion is a liquid preparation obtained by extracting plant-based drugs using hot water at 90°C for 15 minutes. The infusion method was chosen in this study because it is considered simple, affordable, and more closely approximates the general use of traditional medicine, as well as being good for polar and thermostable compounds (Risfianty and Indrawati, 2020).

According to Sevitri's research (2021), it was shown that the concentration of 70% Lamtoro skin extract had the highest inhibitory power (12.9 mm) against *P. acnes* bacteria, then in the research of Sari et al., (2020), it was shown that the antibacterial activity of Lamtoro seed extract had a Minimum Killing Concentration (MBC) against *S. aureus* of 50%.

Based on the potential of lamtoro as an antibacterial, a study was conducted to determine the activity of lamtoro seed infusion against Propionibacterium acnes bacteria at concentrations of 12.5%, 25% and 50%.

2. METHODOLOGY

This research was conducted at the Pharmacognosy Laboratory of the Poltekkes Kemenkes Jayapura and the Microbiology Laboratory of Cenderawasih University in April-May 2025. This research is an experimental laboratory research by testing and observing the inhibitory activity of Lamtoro (*Leucaena leucocephal*a L.) seed infusion extract against Propionibacterium acne bacteria using the diffusion method.

Preparation of Lamtoro Seed Extract: The process was carried out in the Pharmaconosy Lab of the Poltekkes Kemenkes Jayapura. Extraction was carried out using the infusion method using 50 grams of fresh lamtoro seeds with 100 ml of distilled water in a ratio of 1:2. The lamtoro seeds that had been added with distilled water were heated using a water heater for 15 minutes after the temperature in the pan reached 90^{o}C, while stirring occasionally. While hot, they were filtered using a flannel cloth, and 100 ml was made as a stock solution. The stock solution was diluted to 25% and 12.5% concentrations.

Bacterial Sample Preparation: This study was conducted at the UNCE Microbiology Laboratory. A single loop of *P. acnes* bacterial culture from slant agar media was streaked onto sterile Nutrient Agar (NA) media for cultivation. The media was then incubated at 37°C for 18-24 hours. Then the turbidity level was checked by comparing the turbidity of a 0.5 McFarland standard solution (equivalent to 1.5x10^{8} CFU/mL) (Rijal and Asri, 2024). To prepare 38 grams of Muller-Hinton agar (MHA) media in 1000 ml, 2.85 grams of MHA media were dissolved using 75 ml of aquadest. Next, the MHA media was stirred and heated on a hot plate. It was put into 15 petri dishes with 15 ml in each dish and then left at room temperature until it solidified. After that, the MHA media was sterilized in an autoclave for 15 minutes at a temperature of 121^{o}C.

Antibacterial Activity Test of Leucaena Seed Infusion: Four petri dishes containing sterilized MHA media were prepared, then a suspension of *P. acnes* bacteria was taken using a sterile cotton swab and spread evenly on the surface of the agar media. Paper discs were dipped for 15 minutes into leached seed infusion with concentrations of 12.5%, 25% and 50% as well as negative and positive controls. The paper discs that had been dipped into the preparation were then placed on the agar media. The treatment was carried out three times (triplicate), where the first agar media contained three paper discs that had been soaked in 12.5% extract, repeated for the second and third agar media with different concentrations of 25% and 50%. Then the fourth agar media contained one negative control (aquadest) and one positive control (Clindamycin). After that, the media was incubated for 24 hours at 37oC. The inhibition zone formed was measured using a caliper as qualitative data.

3. RESULTS

The seeds of the lamtoro plant were obtained from the Poltekkes Kemenkes campus area in Jayapura. First, the lamtoro seeds were harvested in the morning because the active compound content in the plant tends to be higher at that time. In addition, in the morning the air temperature is still relatively low, thus preventing the evaporation of volatile active compounds (Pantastico, 2020). After harvesting, wet sorting was carried out to select lamtoro seeds that were still green and fresh, then washed using running water with the aim of removing dirt attached to the lamtoro seeds. The washed lamtoro seeds were drained after which an infusion was made.

The following is a table of observations of antibacterial activity tests on Propionibacterium acnes after administering lamtoro seed infusion for 1x24 hours using the disc diffusion method:

Table 1. *Antibacterial Activity Test of Lamtoro Seeds Against Propionibacterium acnes*

Concentration (%)	**Diameter Inhibitor Power**				**Description**
	R1	**R2**	**R3**	**Average (mm)**	
12,5	8,99	7,06	8,52	8,19	Moderate
25	9,29	10,00	9,52	9,60	Moderate
50	9,76	9,59	9,94	9,76	Moderate
K (+) Clindamycin		28,32		28,32	Very Strong
K(-) Aquadest		0		0	-

4. CONCLUSIONS

The lamtoro (*Leucaena leucocephala* L.) plant is a wild plant found almost throughout Indonesia, including Papua. Leucaena consists of small, dense leaves on each branch and also bears fruit. Leucaena fruit contains seeds located transversely within the pods. Leucaena seeds are similar to petai, but smaller (Lailis *et a*l., 2024). Empirically, lamtoro seeds are often used as traditional medicine, including to treat worms, heal wounds, and treat acne. Leucaena seeds contain secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, which have potential antibacterial properties (Sari *et al*., 2020).

The extraction method used was infusion, which involves extracting the herbal medicinal plants using hot water at 90°C for 15 minutes. The infusion method was chosen in this study to prevent damage to the compounds in the samples due to prolonged heating (Risfianty and Indrawati, 2020). The infusion method also has the advantages of being easy to use and requiring simple equipment. Aquadest was chosen as the solvent because it is a polar solvent that is effective in extracting polar compounds from lamtoro seeds, such as flavonoids, alkaloids, tannins, and saponins (Santosa et al., 2023).

The antibacterial activity test of lamtoro seed infusion was conducted to determine its inhibitory power against Propionobacterium acnes bacteria. The first antibacterial activity test was the preparation of a McFarland solution. The McFarland standard was used as a comparison for the number of bacterial colonies in the liquid medium used for antibacterial activity testing, with a certain colony density range. The turbidity of the standard solution was 0.5. The McFarland test corresponds to a cell colony count of approximately 1.5 x 108 CFU/ml (Aviany and Pujiyanto, 2020).

Mueller Hinton Agar (MHA) media was prepared for antibacterial activity testing. This medium is used because all bacteria can grow on it, as it is neither a differential nor a selective medium.

Pathogenic bacteria can grow very easily (Putriani, 2023). Furthermore, this medium is designated as the standard medium for antimicrobial susceptibility testing. Through diffusion, MHA medium plates can create a better antimicrobial diffusion area than most other plates (Fitriana *et al*., 2020).

This test uses the paper disc method. This method is used to test the antimicrobial activity of an antibiotic against disease-causing pathogenic microorganisms. The size of the clear zone formed indicates the sensitivity of the pathogenic microorganism to the antibiotic. The parameter used is the clear zone, which is the clear area around the paper disc, indicating the absence or inhibition of microorganism growth due to the excretion of antimicrobial substances by its competitors (Nurhayati *et al*., 2020).

Bacterial testing was conducted using three concentrations: 12.5%, 25%, and 50%. First, a 50% stock solution was prepared, then diluted to 12.5% , 25%, and 50%. Next, the three samples, along with the positive control (Clindamycin) and negative control (distilled water), were soaked in a petri dish until absorbed. Then, each paper disc was placed in its position in the MHA medium and incubated at 37oC for 1 x 24 hours. Then, the clear zone formed was measured with the aim of creating optimal conditions for the growth of the tested bacteria and to allow the tested antibacterial compounds to diffuse into the growth medium and inhibit bacterial growth (Guntur *et al*., 2021).

In testing the antibacterial activity of lamtoro seed infusion against *P.acnes* bacteria, the results obtained for the concentration of lamtoro seed infusion 12.5% had an inhibitory power of 8.19 mm, including the category of moderate inhibition response, the concentration of 25% had an inhibitory power of 9.60 mm, including the category of moderate inhibition response and the concentration of 50% had an inhibitory power of 9.76 mm, including the category of moderate inhibition response. These results show that the greater the concentration, the higher the inhibition zone produced. This is because the higher the concentration of antimicrobial ingredients will increase the active substances are contained in them, thereby increasing the effectiveness in inhibiting microbes (Angelina *et al*., 2020).

The results of this study align with similar research conducted by Sari *et al*. (2020) on the antibacterial activity of petai cina seed extract against *Staphylococcus aureus*. This study found that a 50% concentration of lamoro seed extract could inhibit *S. aureus*, one of the bacteria that causes acne.

The antibacterial activity of lamtoro seed infusion is due to the chemical compounds contained in the lamtoro seed infusion extract, namely flavonoids and saponins. Where the mechanism of action of flavonoids as antibacterials is by damaging cell walls, deactivating enzyme activity, binding to adhesins, and damaging cell membranes. The beta ring and -OH group in flavonoids are thought to be the structures that play a role in antibacterial activity, while Saponins function as antibacterials by disrupting the integrity and function of bacterial cell walls, namely by causing leakage of proteins and enzymes from within the cell. Saponins can be antibacterial because the active substance on their surface is like a detergent, so saponins will reduce the surface tension of the bacterial cell wall and damage the membrane permeability. Damage to the cell membrane will disrupt the survival of bacteria (Pramesuari, 2023).

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Based on the results of the research that has been carried out, it can be concluded that the antibacterial activity of lamtoro seed infusion with concentrations of 12.5%, 25% and 50% has an average diameter of 8.19 mm, 9.60 mm and 9.76 mm, respectively with a moderate inhibition zone category.

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