

RESEARCH ARTICLE

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Antibacterial activity of ethanol extract of limpasu (*Baccaurea lanceolata*) pericarpium with the ultrasound assisted extraction method against *Propionibacterium acne*

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ABSTRACT

Background: Acne, a prevalent skin condition, can arise from factors such as an unhealthy lifestyle, dietary habits, hormonal imbalances, and bacterial infections. *Propionibacterium acnes* is a notable bacterium responsible for acne. In South Borneo, the pericarpium of the limpasu plant (*Baccaurea lanceolata*) is traditionally used to treat acne.

Objective: This study aims to scientifically validate the empirical antibacterial effects of limpasu pericarpium against *Propionibacterium acnes*, as reported by the communities in South Borneo.

Methods: The ultrasound-assisted extraction (UAE) method was utilized to extract compounds from limpasu pericarpium. The antibacterial activity of the limpasu pericarpium extract was evaluated using the disc diffusion method at concentrations of 20%, 40%, 60%, 80%, and 100% w/v. Clindamycin served as the positive control, and 1% DMSO was the negative control.

Results: The lowest concentration (20%) fell within the strong activity category, producing a clear zone diameter of 18.76 mm. Higher concentrations (40%, 60%, 80%, and 100%) demonstrated more potent antibacterial effects, with inhibition zones of 23.23 mm, 26.06 mm, 26.93 mm, and 27.33 mm, respectively. Notably, 60% to 100% concentrations exhibited greater inhibitory effects than the positive control, clindamycin, which had an inhibition zone of 25.23 mm.

Conclusion: The study confirms the antibacterial properties of limpasu pericarpium against *Propionibacterium acnes*, supporting the traditional claims of its efficacy by the South Borneo communities.

Keywords: limpasu, *Baccaurea lanceolata*, antibacterial, *Propionibacterium acne*

Introduction

Acne is the most common skin infection, affecting 80% to 85% of adolescents in Indonesia, particularly those experiencing puberty. *Propionibacterium acne*, a rod-shaped, anaerobic, and Gram-positive bacterium, plays a significant role in acne development. While *P. acnes* is part of the skin's normal flora, an increase in its population can lead to pore blockage with keratin, causing acne [1]. Acne treatments typically involve

correcting skin follicle abnormalities, reducing sebum production and inflammation, and decreasing the number of *P. acnes* on the skin.

P. acnes can damage the stratum corneum and stratum germinativum by producing lipase, which breaks down triglycerides into free fatty acids. These free fatty acids, combined with skin oils, harden and clog pores, facilitating acne formation. The accumulation of free fatty acids in pores provides a favorable medium for *P. acnes* growth, leading to persistent acne outbreaks [2].

In South Kalimantan, Indonesia, traditional treatments for acne include the use of limpasu fruit peel (*Baccaurea lanceolata*). The peel is finely pounded and applied to acne-prone skin. Limpasu is

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also recognized for its use as a sunscreen due to its natural antioxidant content [3]. Antioxidants are crucial in slowing down the oxidation process of free radicals, which can contribute to skin damage [4].

Limpasu fruit, leaves, and stems exhibit antibacterial activity. The ethanol extract of limpasu fruit peel has demonstrated strong antibacterial effects against various bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and acne-causing bacteria such as *Staphylococcus epidermidis* and *P. acnes* [5]. This antibacterial activity is attributed to secondary metabolites present in limpasu, including alkaloids, phenols, flavonoids, tannins, and saponins [6].

Phenols and flavonoids, which play a key role in the antibacterial activity of limpasu, can be extracted using several methods. Ultrasound-assisted extraction (UAE) and ultrasonic-microwave-assisted extraction (UMAE) are effective techniques for this purpose [7]. UAE, in particular, is noted for its efficiency in obtaining a high yield of flavonoids and other bioactive compounds [8]. UAE can increase tannin yield by 17.16% [9] and is also effective for extracting saponins [10]. This study aims to determine the antibacterial activity of limpasu fruit peel extract, obtained through UAE, against *P. acnes*. By exploring the efficacy of traditional treatments combined with modern extraction techniques, we seek to validate and potentially enhance the use of limpasu in acne treatment.

Method

Preparation of simplisia

A total of 15 kg of limpasu (*Baccaurea lanceolata*) was wet sorted, washed thoroughly with running water, and drained. The limpasu fruit peel was thinly chopped and aerated in an air-conditioned room for one day. Drying was continued using an oven dryer at 50°C until dry simplisia was obtained. The simplisia was then pulverized and sieved using a 40-mesh sieve.

Evaluation of simplisia quality

Moisture content: 10 g of limpasu fruit peel simplisia was placed in a pre-filled vaporizer cup, dried in an oven (105°C) for 5 hours, and then weighed. Drying and weighing were repeated at 1-hour intervals until the difference between two consecutive weighings did not exceed 0.25% [11].

Drying shrinkage: 1 g of limpasu fruit peel simplisia was flattened in a porcelain cup and dried in an oven

(105°C) with the porcelain cup, then weighed. This process was repeated until a stable weight was achieved. The maximum limit of drying shrinkage is 11% [12].

Water soluble content: 5 g of limpasu fruit peel simplisia was placed in a flask with a lid, and 100 mL of chloroform-saturated water was added. The mixture was shaken for the first 6 hours and then allowed to stand for 18 hours. The liquid was filtered, and 20 mL of the filtrate was taken, evaporated, dried in an oven (105°C), and weighed. This process was repeated until a stable weight was obtained [11].

Ethanol soluble content: 5 g of limpasu fruit peel simplisia was placed in a flask with a lid, and 100 mL of ethanol pro analysis was added. The mixture was shaken for the first 6 hours and then left to stand for 18 hours. The liquid was quickly filtered, and 20 mL of the filtrate was evaporated and dried in an oven (105°C). This process was repeated until a stable weight was obtained [11].

Preparation of limpasu fruit peel extract

A total of 840 g of limpasu fruit peel simplisia powder was extracted with 70% ethanol at a ratio of 1:7 in a 500 mL Erlenmeyer flask covered with aluminum foil. The flask was placed in an ultrasonic water bath (Biobase) and extracted for 30 minutes at an ultrasonic frequency of 40 kHz. The liquid was filtered, and the filtrate was evaporated alternately using a water bath during the day and aerated in an air-conditioned room overnight for approximately two weeks until a thick extract was obtained.

Phytochemical screening

Preparation of test solution: 500 mg of limpasu fruit peel extract was dissolved in 50 mL of 70% ethanol until homogeneous. This test solution was used for phytochemical screening of several secondary metabolites [13].

Alkaloid test: 2 mL of the test solution was evaporated in a porcelain cup. The residue was dissolved with 6 mL of 2 N HCl, then placed into a test tube, and a drop of Dragendorff reagent was added. Positive results were indicated by the formation of an orange or brick-red precipitate [14].

Phenol test: 2 mL of the test solution in a test tube was treated with FeCl₃ 10%. Positive results were indicated by a color change to dark blue or greenish black [15].

Flavonoid test: 1 mL of the test solution was added to 1 g of magnesium powder and 1 mL of concentrated HCl. Positive results were indicated by a yellow-orange color [14].

Tannin test: 2 mL of the test solution in a test tube was mixed with 2 mL of 1% gelatin solution. Positive results were indicated by the formation of a white precipitate [16].

Saponin test: A small amount of the extract was placed in a test tube, and aquadest was added and shaken vigorously for 10 seconds. If foam formed to a height of 1-10 cm and remained stable for the next 10 minutes, then the foam was treated with 2 N HCl. If the foam remained stable, the extract was considered positive for saponins [13].

Inoculation: Inoculation was carried out on previously prepared slant agar media. The process involved transferring one cotton-bud *P. acnes* swab from pure culture onto the inclined media by streaking the agar surface, followed by incubation for 24 hours at 37°C [17].

Preparation of *P. acnes* suspension: *P. acnes* incubated on the agar was collected using an inoculation loop and dissolved in 10 mL of 0.9% NaCl solution. The turbidity of the bacterial suspension was visually compared with McFarland 0.5 standard solution (1.5×10^8 CFU/mL) [18].

Antibacterial Activity Test

This test was conducted using the disc diffusion technique to measure the antibacterial activity of the extracts applied to blank discs. The test was performed in three replications (triplicate). Preparation of limpasu fruit peel extract concentration Series (% b/v):

- 20% concentration: 1 g of ethanol extract of limpasu fruit peel dissolved in 1% DMSO to a total volume of 5 mL.
- 40% concentration: 2 g of limpasu fruit peel ethanol extract dissolved in 1% DMSO to a total volume of 5 mL.
- 60% concentration: 3 g of limpasu fruit peel ethanol extract dissolved in 1% DMSO to a total volume of 5 mL.
- 80% concentration: 4 g of limpasu fruit peel ethanol extract dissolved in 1% DMSO to a total volume of 5 mL.
- 100% concentration: 5 g of limpasu fruit peel

ethanol extract dissolved in 1% DMSO to a total volume of 5 mL.

Nine Petri dishes containing nutrient agar (NA) were prepared, and the bacteria were spread using the swabbing method with the bacterial suspension, then left to stand for about 10-15 minutes. Discs with the concentration series of ethanol extracts of limpasu fruit peel, negative control discs soaked in 1% DMSO, and positive control discs (clindamycin 2.13 µg/disc) were placed on the surface of the NA using tweezers. The dishes were incubated for 12 hours at 37°C. After incubation, the clear zones on the Petri dishes were measured using a digital caliper, with the diameter of the clear zones measured in millimeters [19].

Statistical analysis

The data obtained were analyzed using Microsoft Excel 2019 software.

Results

Simplisia preparation and quality evaluation

The preparation of limpasu fruit peel simplisia from 15 kg of raw samples produced 1200 g of simplisia powder, yielding 8%. The quality evaluation of simplisia included four tests: two non-specific and two specific parameters. Non-specific parameters included moisture content and drying shrinkage, measured using the gravimetric method, which yielded values of 6.87% and 7%, respectively (Table 1). These results comply with the applicable requirements of a maximum moisture content of 10% and a maximum drying shrinkage limit of 11%.

Table 1. Quality evaluation results of limpasu fruit peel simplisia

Parameter	Value	Requirement
Moisture content	6.87%	<10%
Drying shrinkage	7%	<11%
Water soluble content	19.68%	>18%
Ethanol soluble content	19.88%	>12.5%

Preparation of limpasu fruit peel extract and phytochemical screening

Extraction was performed using the ultrasound-assisted extraction (UAE) method, yielding 212.21 g of a viscous limpasu fruit peel extract, with a yield value

Table 2. Phytochemical screening results of limpasu fruit peel extract

Secondary metabolite	Reagent	Result	Interpretation result
Alkaloids	Dragendroff	Brick red precipitate	Positive
Phenols	FeCl ₃	Solution turns blackish green	Positive
Flavonoids	Magnesium powder and concentrated HCl	Solution turns yellow-orange	Positive
Tannins	Gelatin 1% solution	White precipitate	Positive
Saponins	Water and HCl 2 N	Foam formed	Positive

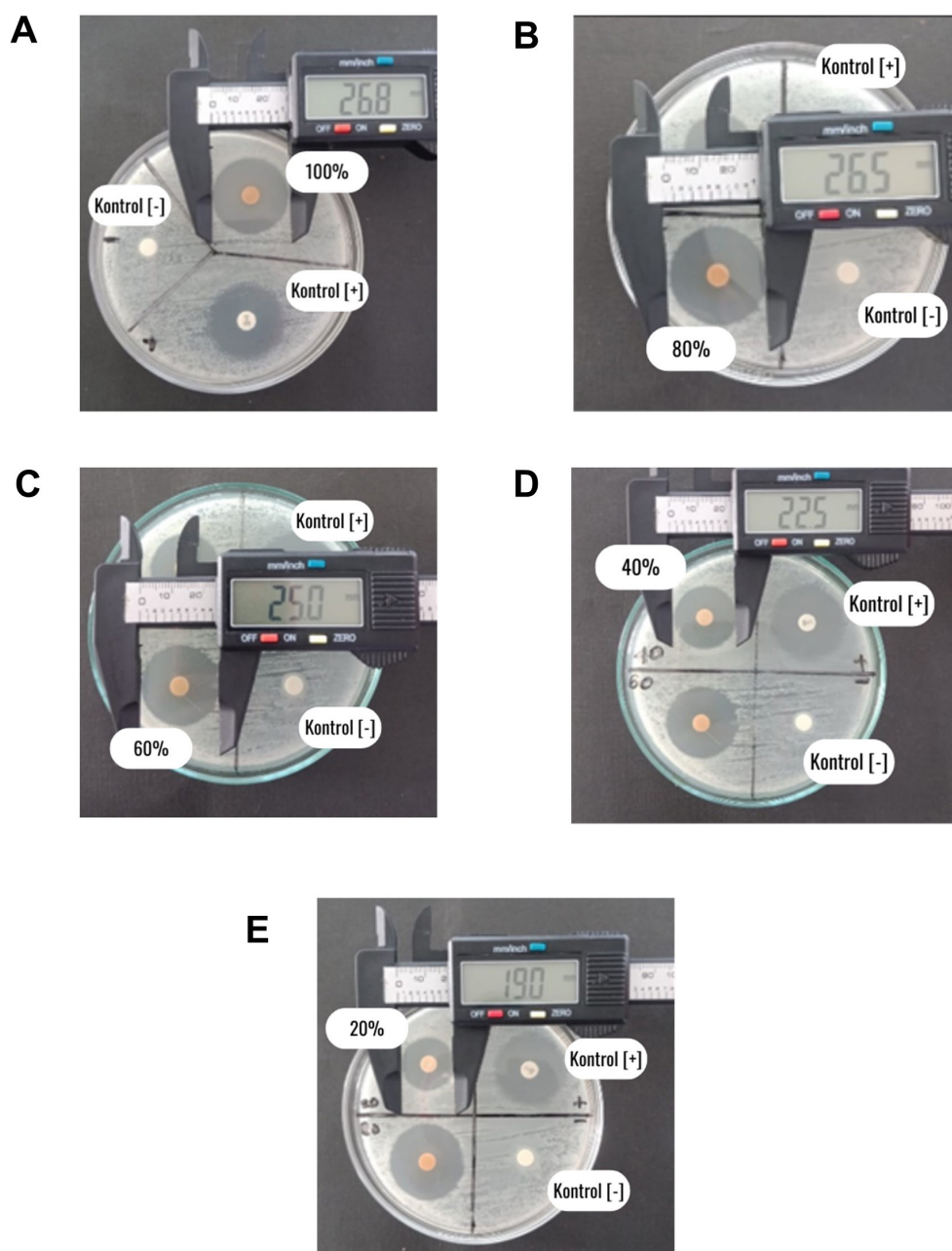


Figure 1. Clear zone produced by limpasu fruit peel extract on *P. acnes* growth media. (a) 100% concentration, (b) 80% concentration, (c) 60% concentration, (d) 40% concentration, (e) 20% concentration. The brown disks represent the series of concentrations (20%-100%). The white disk with a clear zone around it represents the positive control (clindamycin), while the white disk with no clear zone around it represents the negative control (DMSO 1%).

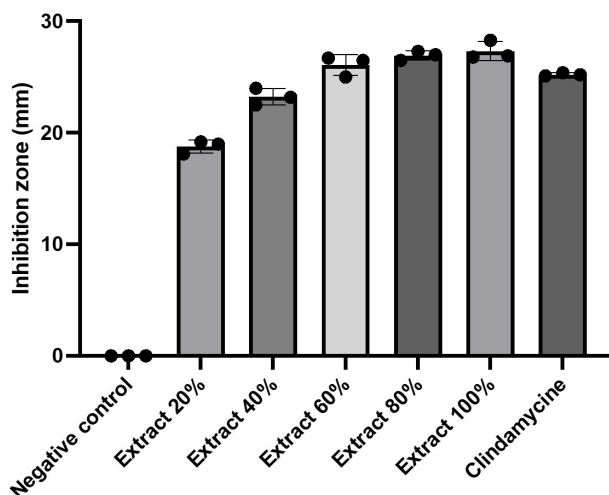


Figure 2. Diameter of inhibition zone of ethanol extract of limpasu fruit peel against *P. acnes*

of 25.26%. Phytochemical screening of the limpasu fruit peel extract revealed the presence of several secondary metabolites (Table 2).

Antibacterial test results

The antibacterial activity test revealed that the 20% extract concentration exhibited strong inhibition, with an average clear zone diameter of 18.76 mm. Higher concentrations showed very strong inhibition against *P. acnes*, with the 40% concentration resulting in a 23.23 mm clear zone, the 60% concentration yielding a 26.06 mm clear zone, the 80% concentration producing a 26.93 mm clear zone, and the 100% concentration creating a 27.33 mm clear zone. Notably, the 60%, 80%, and 100% concentrations demonstrated greater antibacterial efficacy than the clindamycin positive control, which had an average diameter of 25.23 mm (Figures 1 and 2).

Discussion

The positive control was a clindamycin antibiotic disk obtained from the Faculty of Medicine, ULM Banjarbaru. Clindamycin is known for its effectiveness in treating skin infections, especially those caused by *P. acnes* [20]. Previous research has demonstrated that ethanol extract from limpasu fruit is effective against *Escherichia coli*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* [5]. Furthermore, extracts from the leaves and stems of limpasu plants have shown antibacterial activity, though not antifungal activity, with moderate to strong effects on *E. coli* (7 mm), *S. enterica* (7.3 mm), *S. pyogenes* (7.6 mm), *S. aureus* (8 mm), *P. aeruginosa*

(8.3 mm), *B. cereus* (12 mm), and *M. catarrhalis* (12 mm) [21]. This antibacterial activity test provides scientific validation for the empirical use of limpasu fruit peel by people in South Kalimantan as an acne treatment.

Conclusion

Limpasu fruit peel extract exhibits significant antibacterial activity against *P. acnes*. Although this study has not yet determined the minimum inhibitory concentration (MIC), the lowest extract concentration of 20% demonstrated strong antibacterial ability, while concentrations ranging from 40% to 100% exhibited very strong antibacterial activity. These findings support the traditional use of limpasu fruit peel as an effective natural treatment for acne.

Acknowledgment

None.

Conflict of interest

None.

Author contributions

RU played a role in designing the study, collecting data, and drafting the first version of the manuscript. SN and IZ contributed to data analysis. All authors contributed to data interpretation and approved the final version of the manuscript.

Received: June 16, 2022

Revised: April 4, 2024

Accepted: April 19, 2024

Published online: May 13, 2024

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