

Antibacterial activity test of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*

Maria Fransiska Priska Yulianti¹, Anita Lidesna Shinta Amat², Regina Marvina Hutasoit², Prisca Deviani Pakan^{3*}

¹Medicine Program, Faculty of Medicine and Veterinary Science, Universitas Nusa Cendana, Kupang City, 85001, Indonesia

²Department of Biomedicine of Medicine Program, Faculty of Medicine and Veterinary Science, Universitas Nusa Cendana, Kupang City, 85001, Indonesia

³Department of Pharmacology of Pharmacy Program, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, 85001, Indonesia

*Corresponding author: Medicine Program, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Jalan Adisucipto Penfui, Kupang City, Indonesia, 85001. Email: prischayu19@email.com

ABSTRACT

Background: *Propionibacterium acnes* is a bacterium found on the skin that plays a significant role in acne vulgaris. The inappropriate use of antibiotics can lead to resistance, necessitating the search for alternative therapies from plants with high antibacterial potential. One such plant is jamblang (*Syzygium cumini*), which has demonstrated antibacterial properties.

Objective: This research aims to test the antibacterial activity of jamblang leaf extract against the growth of *P. acnes*.

Method: The study employed an experimental design with a posttest-only control group design. The treatment groups consisted of a positive control (clindamycin), a negative control (sterile distilled water), and jamblang leaf extract groups with concentrations of 100%, 50%, 25%, 12.5%, and 6.25% (n=4). Data analysis was conducted using the One-Way ANOVA statistical test.

Results: The jamblang leaf extract exhibited antibacterial potential against the growth of *P. acnes*. The extract showed strong antibacterial activity at concentrations of 100%, 50%, 25%, and 12.5%, and moderate antibacterial activity at a concentration of 6.25%.

Conclusion: This suggests that jamblang leaf extract could be a promising alternative therapy for treating acne vulgaris, offering a natural solution to combat antibiotic resistance.

Keywords: *Propionibacterium acnes*, acne vulgaris, jamblang leaf extract, antibacterial activity, *Syzygium cumini*

Introduction

Human skin is one of the most important parts of the human body because it provides critical protection against various environmental exposures such as bacteria, viruses, and physical damage. Additionally, it regulates body temperature and enables tactile sensations. The skin also harbors a multitude of microorganisms [1]. Among these is *Propionibacterium acnes*, an anaerobic, Gram-positive bacterium commonly found in the sebaceous regions of human skin. This bacterium can reach the skin surface via the sebum flow. While *P. acnes* is generally

non-pathogenic under normal conditions, it can become invasive when skin conditions change. It produces lipase enzymes that break down free fatty acids from skin lipids, leading to tissue inflammation [2].

One of the most prevalent skin conditions caused by *P. acnes* is acne. The condition exacerbates when there is an excess of triglycerides in sebum, providing nutrients for these bacteria. *P. acnes* contributes to acne inflammation by producing chemotactic factors and lipase enzymes, which convert triglycerides into free fatty acids and

activate both classical and alternative complement pathways [3].

Acne affects over 80% of the population and can occur at all ages. According to the Global Burden of Disease (GBD) study, acne is the eighth most common skin disease worldwide, affecting 85% of young adults aged 12 to 25. Almost everyone experiences acne at some point, particularly during adolescence, with an incidence rate of approximately 85%. The highest prevalence is observed in females aged 14-17 years (83-85%) and males aged 16-19 years (95-100%). Surveys in Southeast Asia report acne cases ranging from 40-80% [4,5,6].

In Indonesia, acne ranks as the third most frequent skin disease in dermatology and venereal disease departments of hospitals and clinics, according to the 2015 Cosmetic Dermatology Study. The Indonesian Aesthetic Dermatology Research indicates a yearly increase in acne cases, with a survey showing a rise from 60% in 2006 to 80% in 2007, and 90% in 2009. While acne is not fatal, it significantly impacts self-confidence and quality of life [5,6].

Acne treatment includes systemic and topical therapies. Antibiotics are often used to treat bacterial infections causing acne. However, the incidence of antibiotic resistance is increasing. Studies from various countries report that over 50% of *P. acnes* strains are resistant to topical macrolides, making these treatments less effective [7]. Tetracycline is the most commonly used antibiotic therapy for acne, but its effectiveness is compromised due to high resistance rates. A study at Hasan Sadikin Hospital in Bandung, Indonesia, reported *P. acnes* resistance rates of 16% for tetracycline, 32% for erythromycin, and 43% for clindamycin [7]. Inappropriate antibiotic use contributes to this resistance, highlighting the need for alternative therapies, such as plant-based antibacterials [7,8].

Jamblang (*Syzygium cumini*), a plant belonging to the guava family (Myrtaceae), has been used in traditional medicine. In Indonesia, it is known by various names, including jambe kleng (Aceh), jujutan and juwet (Bali), and jamblang (Betawi and Sunda). Previous studies have shown that jamblang

leaf extract contains phenols, flavonoids, terpenoids, tannins, saponins, and steroids, which have proven antibacterial activity. Ethanol extracts of jamblang leaves have demonstrated antimicrobial effects against both Gram-positive and Gram-negative bacteria [9,10,11].

Despite the numerous studies on the jamblang plant, research on the effectiveness of its antibacterial properties, specifically against *P. acnes*, is limited. Therefore, this study aims to evaluate the activity of jamblang leaf extract against *P. acnes*.

Methods

Sample collection

Fresh, green jamblang leaves (*Syzygium cumini*) were collected from Jl. Sam Ratulangi Raya No.3, Kec. Kelapa Lima, Kel. Oesapa Barat, Kupang City, East Nusa Tenggara (NTT). The bacterial strain used in this study was *Propionibacterium acnes*, obtained from the Surabaya Health Laboratory Center. The study included seven groups: five groups of jamblang leaf extract with concentrations of 100%, 50%, 25%, 12.5%, and 6.25%, a negative control group using sterile distilled water, and a positive control group using clindamycin (n=4).

Jamblang leaf extraction

Five kilograms of jamblang leaves were cleaned and dried by aerating without sunlight for four days. The dried leaves were then pulverized using a blender and macerated in 70% ethanol at a 1:5 ratio for three days with daily stirring. After soaking, the mixture was filtered using filter paper to obtain 2 liters of liquid extract. This liquid extract was then evaporated using a vacuum rotary evaporator to produce a thick jamblang leaf extract.

Ethanol free test and phytochemical test

An ethanol-free test was performed by reacting potassium dichromate ($K_2Cr_2O_7$) with ethanol in an acidic environment. Phytochemical tests on the jamblang leaf extract included examinations for alkaloids, terpenoids, flavonoids, saponins, and tannins.

Bacterial confirmation test

A bacterial confirmation test was performed using Gram staining. The process involved: (i) initial staining: crystal violet dye was applied to the slide, (ii) Mordant application: iodine was added to fix the dye, (iii) decolorization, ethanol was used to remove the dye from some bacteria, (iv) counterstaining: safranin was applied to stain decolorized bacteria pink. Gram-positive bacteria retained the crystal violet dye, appearing purple, while Gram-negative bacteria took up the safranin and appeared pink.

Antibacterial test

The antibacterial activity was tested using the Kirby-Bauer disk diffusion method. A sterile cotton swab was dipped into a *P. acnes* bacterial suspension and spread evenly on nutrient agar (NA). After a 10-minute absorption period, 6 mm diameter paper discs were dipped into each concentration of jamblang leaf extract for 30 minutes and then placed on the agar plates using sterile tweezers. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured with a caliper.

Data analysis

The inhibition zones were categorized as follows: ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), ≥ 20 mm: very strong. The study employed a true experimental design with a posttest-only control group design. Bivariate data analysis was conducted using a One-Way ANOVA test, followed by a post hoc test with the Dunnett T3 test.

Results

Jamblang leaf extraction and phytochemical test

A total of 5 kg of jamblang leaves yielded 191 grams of thick extract. The mixed-colored reaction of the extract with $K_2Cr_2O_7$, and H_2SO_4 indicated the absence of ethanol in the extract. Phytochemical test results revealed that the

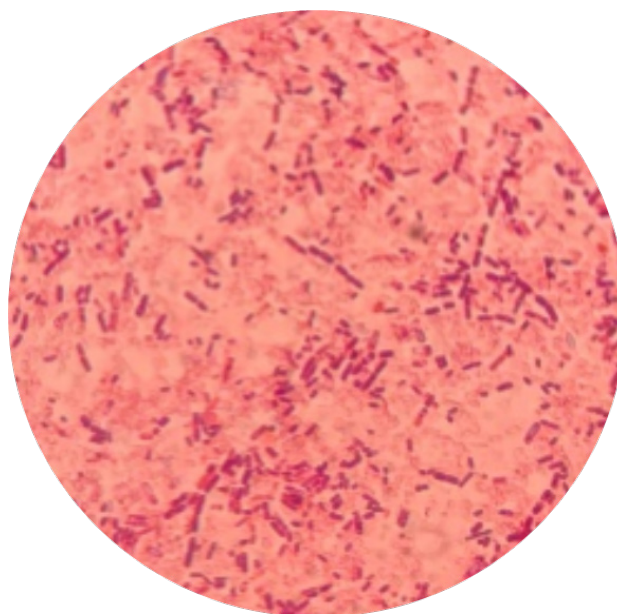


Figure 1. Gram staining results showed *P. acnes*

jamblang leaf extract contains several secondary metabolite compounds with antibacterial properties, namely alkaloids, terpenoids, flavonoids, saponins, and tannins.

Antibacterial test

Gram staining of *P. acnes* showed purple rod-shaped bacteria, confirming them as Gram-positive (Figure 1). The antibacterial activity of jamblang leaf extract was tested on *P. acnes* at concentrations of 100%, 50%, 25%, 12.5%, and 6.25%. The results demonstrated that the jamblang leaf extract inhibited the growth of *P. acnes* in a concentration-dependent manner, with higher concentrations producing larger inhibition zones (Figure 2, Table 1).

The data were analyzed using the One-Way ANOVA test and the Post Hoc Dunnett T3 test. The normality test indicated that the data distribution was normal (p -value > 0.05). The One-Way ANOVA test yielded a p -value of 0.000, which is less than the significance level ($\alpha = 0.05$), leading to the rejection of the null hypothesis (H_0) and acceptance of the alternative hypothesis (H_1). This confirms that there is a significant difference in the average diameter of the inhibition zones between the treatment groups.

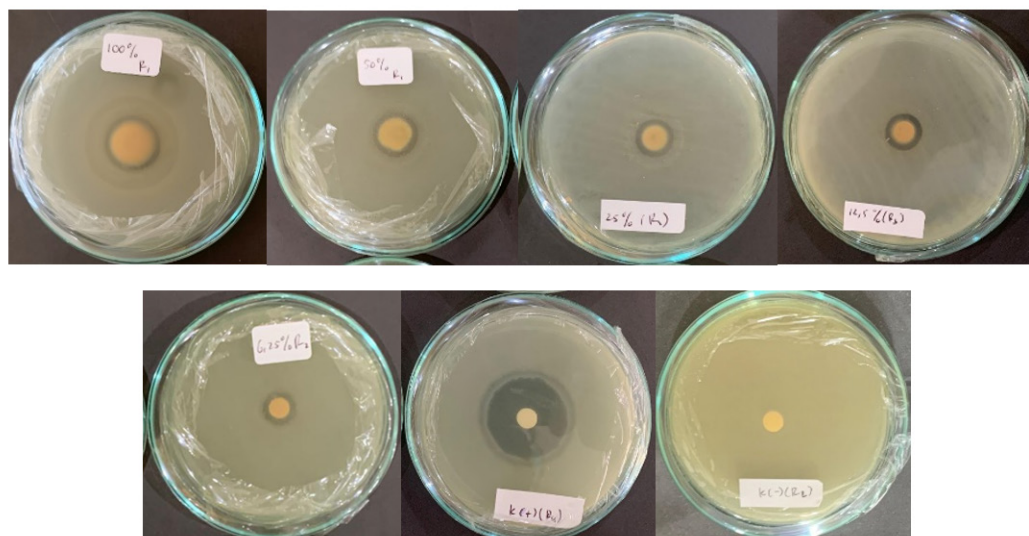


Figure 2. Antibacterial test results of jamblang leaf extract on *Propionibacterium acnes*

Table 1. The results of measuring the average diameter of the inhibition zone of jamblang leaf extract on the growth of *P. acnes*

Treatment	Average inhibition zone diameter (mm)	Potential
Clindamycin	25.90	Very strong
Extract 100%	19.01	Strong
Extract 50%	15.24	Strong
Extract 25%	11.83	Strong
Extract 12.5%	10.98	Strong
Extract 6.25%	9.59	Medium
Distilled water	0	Weak

Discussion

This study aimed to evaluate the antibacterial activity of jamblang leaf extract on the growth of *Propionibacterium acnes* by measuring the inhibition zone around the disc, indicated by the presence of a clear zone in the test agar medium. The findings revealed that jamblang leaf extract exhibits antibacterial activity against *P. acnes* at concentrations of 100%, 50%, 25%, and 12.5%, with strong antibacterial potential, while a concentration of 6.25% demonstrated moderate potential. The One-Way ANOVA test results showed a significant mean difference in the inhibition zone diameters among the different concentrations of jamblang leaf extract.

The size of the inhibition zone increased with higher concentrations of jamblang leaf extract. This can be attributed to the greater presence

of secondary metabolic compounds in the extract at higher concentrations. As the concentration increases, more antibacterial compounds are released, enhancing their penetration into bacterial cells and resulting in larger inhibition zones at 100% concentration [16].

The positive control, clindamycin, exhibited very strong antibacterial activity, forming a large inhibition zone. Clindamycin is effective against Gram-positive bacteria and functions by binding to the 50S subunit of bacterial ribosomes, thereby inhibiting protein synthesis. This study confirmed the potent antibacterial activity of clindamycin, as evidenced by the significant inhibition zone formed in the positive control group.

Jamblang leaf extract demonstrated antibacterial potential due to the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins,

and tannins. Flavonoids damage the cytoplasmic membrane, causing leakage of essential metabolites and inactivation of bacterial enzymes. Alkaloids interfere with the peptidoglycan component of bacterial cell walls, leading to incomplete cell formation and increased susceptibility to lysis. Terpenoids damage bacterial cell membranes and penetrate the cell walls due to their lipid-soluble properties. Triterpenoids react with porins on the bacterial outer membrane, forming strong polymer bonds that reduce cell wall permeability. Saponins increase bacterial cell permeability, causing cell hemolysis. Tannins denature proteins and inhibit bacterial digestion [12].

The antibacterial activity of jamblang leaf extract aligns with previous studies, which also identified the presence of alkaloids, triterpenoids, flavonoids, saponins, and tannins in the extract [13]. Previous research demonstrated that jamblang leaf extract inhibits the growth of *Staphylococcus epidermidis*, another Gram-positive anaerobic bacterium [15]. The diameter of the inhibition zone increased with higher concentrations of jamblang leaf extract, consistent with the findings of this study. Furthermore, polyherbal anti-acne gel containing mango seed extract and jamblang showed antibacterial properties against *P. acnes* [14,15].

In summary, jamblang leaf extract has proven antibacterial activity against *P. acnes*, attributed to its secondary metabolite content. Further research is recommended to explore its efficacy against other pathogenic bacteria, potentially offering a natural antibacterial solution for *P. acnes* infections and benefiting the broader community.

Conclusion

Jamblang leaf extract demonstrates antibacterial activity against the growth of *P. acnes*. Concentrations of 100%, 50%, 25%, and 12.5% show strong antibacterial potential, while a concentration of 6.25% exhibits moderate potential.

Acknowledgment

We are grateful to the Faculty of Medicine and Veterinary Sciences, Universitas Nusa Cendana, for

providing the necessary resources and support to conduct this research.

Funding

None.

Declaration of interest

None.

Author contributions

M.F.P.Y. performed experiments, collected the research data, and wrote the manuscript, A.L.S.A., R.M.H, P.D.P. supervised study, contributed to design, and helped the completion of the manuscript.

Received: April 4, 2024

Revised: April 26, 2024

Accepted: May 14, 2024

Published online: July 9, 2024

References

1. Sinaulan CD, Hantara A. Model Klasifikasi Permasalahan Kulit Wajah Menggunakan Metode Support Vector Machine. *J Pendidik dan Kewirausahaan*. 2021;9(1):297-308. <https://doi.org/10.47668/pkwu.v9i1.246>
2. Wulandari A, Farida Y, Taurhesia S. Perbandingan Aktivitas Ekstrak Daun Kelor Dan Teh Hijau Serta Kombinasi Sebagai Antibakteri Penyebab Jerawat. *J Fitofarmaka Indones*. 2020;7(2):23-9. <https://doi.org/10.33096/jffi.v7i2.535>
3. Indarto I, Narulita W, Anggoro BS, Novitasari A. Aktivitas Antibakteri Ekstrak Daun Binahong Terhadap *Propionibacterium acnes*. *Biosf J Tadris Biol*. 2019;10(1):67-78. <https://doi.org/10.24042/biosfer.v10i1.4102>
4. Lynn D, Umari T, Dellavalle R, Dunnick C. The epidemiology of acne vulgaris in late adolescence. *Adolesc Health Med Ther*. 2016;13. <https://doi.org/10.2147/AHMT.S55832>
5. Sinaga F. Gambaran Pemakaian Kosmetik Pada Pasien Akne Vulgaris Di Poliklinik Kulit Dan Kelamin Rsu Royal Prima Dan Murni Teguh Memorial Hospital Kota Medan. *Nommensen J Med*. 2022;8(1):10-3. <https://doi.org/10.36655/njm.v8i1.626>
6. Sifatullah N, Zulkarnain Z. Jerawat (Acne vulgaris): Review penyakit infeksi pada kulit. *Pros Semin Nas Biol* [Internet]. 2021;(November):19-23.
7. Zahrah H, Mustika A, Debora K. Aktivitas Antibakteri dan Perubahan Morfologi dari *Propionibacterium acnes* Setelah Pemberian Ekstrak Curcuma Xanthorrhiza. *J Biosains*

- Pascasarj. 2019;20(3):160. <https://doi.org/10.20473/jbp.v20i3.2018.160-169>
8. Susanti G, Asrul M, Tusnani S. Pemanfaatan Ekstrak Daun Lerek sebagai Antibakteri terhadap Bakteri Penyebab Jerawat *Propionibacterium acnes*. *Jumantik (Jurnal Ilm Penelit Kesehatan)*. 2022;7(2):94. <https://doi.org/10.30829/jumantik.v7i2.10455>
 9. Rahmitasari RD, Suryani D, Hanifa NI. Aktivitas Antibakteri Ekstrak Etanolik Daun Juwet (*Syzygium cumini* (L.) Skeels) terhadap Bakteri Isolat Klinis *Salmonella typhi*. *Pharm J Farm Indones (Pharmaceutical J Indones)*. 2020;17(1):138. <https://doi.org/10.30595/pharmacy.v17i1.6448>
 10. Sudarmi K, Darmayasa IBG, Muksin IK. Uji Fitokimia Dan Daya Hambat Ekstrak Daun Juwet (*Syzygium cumini*) Terhadap Pertumbuhan *Escherichia coli* Dan *Staphylococcus aureus* ATCC. *Simbiosis J Biol Sci*. 2017;5(2):47. <https://doi.org/10.24843/JSIMBIOSIS.2017.v05.i02.p03>
 11. Naim M, Hisani W. Identifikasi dan Karakterisasi Jenis Juwet (*Syzygium cumini*) pada Berbagai Daerah di Sulawesi Selatan. *J Perbal Fak Pertan Univ Cokroaminoto Palopo*. 2018;6(3):76-88.
 12. Dwicahyani T, Sumardianto S, Rianingsih L. Uji Bioaktivitas Ekstrak Teripang Keling (*Holothuria Atra*) Sebagai Antibakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan [Online]*. 2018 Apr;7(1):15-24.
 13. Munira M, Zakiah N, Handayani R, Nasir M. Potensi Antimikroba Ekstrak Daun Jambalang (*Syzygium cumini* L.) Dari Kawasan Geothermal Ie Seum Aceh Besar. *J Insa Farm Indones*. 2022;5(1):98-107 <https://doi.org/10.36387/jifi.v5i1.915>
 14. Agarwal P, Tyagi N, Gaur PK, Puri D, Shanmugam SK. Polyherbal antiacne Gel Containing Extracts of *Mangifera indica* and *Syzygium cumini* seeds: Bioassay Guided Activity Against *Propionibacterium acne*. *Biointerface Res Appl Chem*. 2019;9(4):4177-82. <https://doi.org/10.33263/BRIAC94.177182>
 15. Rachmawati N, Maulidiyah G, Aminah. Uji Daya Hambat dan Toksisitas Ekstrak Daun Jambalang [*Syzygium cumini* (L.) Skeels] Terhadap Pertumbuhan Bakteri *Staphylococcus epidermidis*. *J Biol Indones*. 2021;17(1):39-46. <https://doi.org/10.47349/jbi/17012021/39>
 16. Emelda, Safitri, Fatmawati. Aktivitas Inhibisi Ekstrak Etanolik *Ulva lactuca* terhadap Bakteri *Staphylococcus aureus*. *Pharm J Indones*. 2021;7(1):44. <https://doi.org/10.21776/ub.pji.2021.007.01.7>