

Antibacterial and Biofilm Formation Inhibitory Activities of Kratom (*Mitragyna speciosa* Korth.) Leaf Extract Against *Cutibacterium acnes*

Ramayta Azizah ¹, Henra Henra ^{2,3,4}, Hasyrul Hamzah ^{1,2,*}, Nur Atika Astriani ^{2,5},
Sajida Khairallah Divatunnisa ¹, Nita Talia ¹, and Suhardiansyah ^{1,2}

¹ Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda 75124, Indonesia

² Borneo Bioinfection Research Center, Samarinda, Kalimantan Timur 75124, Indonesia

³ Doctoral Program of Agricultural Science, Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora No 1, Bulaksumur, Yogyakarta 55281, Indonesia

⁴ Agrotechnology Study Program, Faculty of Agriculture, STIPER Agricultural Institute, Jl. Nangka II, Krodan, Maguwoharjo, Yogyakarta 55281, Indonesia

⁵ Master's Program Pharmaceutical Sciences, Faculty of Pharmacy Universitas Gadjah Mada, Jl. Sekip Utara, Yogyakarta 55281, Indonesia

ABSTRACT

Kratom (*Mitragyna speciosa* Korth.) is a tropical plant native to Southeast Asia and has demonstrated antibacterial activity; however, information regarding its activity against biofilm formation by *Cutibacterium acnes* remains limited. This study aimed to evaluate the antibacterial and biofilm formation inhibitory activities of ethanolic kratom leaf extract against *C. acnes*. Antibacterial activity was evaluated using the disc diffusion assay. Biofilm formation inhibition was assessed using a microtiter plate crystal violet assay with optical density measurement at 595 nm. The extract was tested at 1%, 0.5%, 0.25%, and 0.125% (w/v). The 1% extract produced the largest antibacterial inhibition zone among the extract treatments (10.8 mm). In the biofilm assay, the 1% extract produced 70.67% inhibition after 24 h and 58.75% after 48 h. The lowest tested concentration producing at least 50% biofilm inhibition was 0.5% (w/v) at both time points. These findings indicate concentration-related antibacterial and biofilm formation inhibitory activities of kratom leaf extract against *C. acnes*. The specific active compounds responsible for these effects remain to be identified.

Keywords: *Cutibacterium acnes*, antibacterial, biofilm formation inhibition, kratom leaf, *acne*.

INTRODUCTION

Mitragyna speciosa Korth. (kratom) is a tropical member of the Rubiaceae that is widely distributed in Southeast Asia, including Indonesia, Thailand, Malaysia, Myanmar, and

the Philippines (Firmansyah *et al.*, 2021). Kratom leaves contain several classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, glycosides, and saponins (Yuniarti *et al.*, 2020; Masriani *et al.*, 2023). Mitragynine is one of the major alkaloids

*Corresponding author: Hasyrul Hamzah (email: hh241@umkt.ac.id)

DOI: 10.14203/ann.bogor.2022.v30.n1.32-41

Received: August 18, 2026; Revised: September 12, 2026; Accepted: September 13, 2026

Available online: September 30, 2026; Published: September 30, 2026

ISSN 0517-8452 (print) | 2407-7518 (online)

Annals Bogorienses is published by BRIN Publishing. This is an open access article Creative Common CC-by-SA License (<https://creativecommons.org/licenses/by-sa/4.0/>)

reported in kratom leaves (Firmansyah *et al.*, 2021; Sudarwati, 2021). Previous studies have reported antibacterial activity of kratom preparations, supporting further investigation of this plant as a source of antimicrobial compounds (Suhaimi *et al.*, 2019; Puspasari & Krismonika, 2020).

Acne vulgaris is a multifactorial inflammatory disorder of the pilosebaceous unit involving altered follicular keratinization, sebum production, host inflammatory responses, and changes in the skin microbiome. *Cutibacterium acnes* is a common skin commensal, and acne is not explained simply by an increased total abundance of this organism. Rather, disease-associated phylotypes, microbiome imbalance, host responses, and biofilm formation may contribute to inflammation and persistence (Dréno *et al.*, 2018; Coenye *et al.*, 2022). Biofilm growth can increase bacterial tolerance to antimicrobial treatment and may therefore represent a relevant target for anti-acne strategies (Coenye *et al.*, 2022).

Plant-derived extracts are of interest as potential sources of antibacterial and biofilm-modulating agents, and several plant-derived preparations have shown activity against acne-associated bacteria and biofilms (De Canha *et al.*, 2020). However, qualitative detection of a phytochemical class does not establish that the detected compounds are responsible for a biological effect. For kratom in particular, any relationship between the observed activity and specific alkaloids, flavonoids, phenolics, or other constituents requires chemical characterization, fractionation, or testing of purified compounds (Yuniarti *et al.*, 2020; Masriani *et al.*, 2023).

Previous studies have reported antibacterial activity of kratom preparations against acne-associated bacteria. Suhaimi *et al.* (2019) reported antibacterial activity of kratom leaf extract against *C. acnes*, while Puspasari and Krismonika (2020) reported inhibition of *Staphylococcus epidermidis* by kratom leaf extract. Putri *et al.* (2023) also demonstrated antibacterial activity of kratom root and stem extracts against *C. acnes*. In contrast, evidence regarding the effect of kratom leaf extract on *C. acnes* biofilm formation remains limited. Therefore, this study aimed to evaluate the antibacterial activity and inhibition of biofilm

formation by ethanolic kratom leaf extract against *C. acnes*.

MATERIALS AND METHODS

Research Design

This in vitro experimental study evaluated the antibacterial activity and inhibition of biofilm formation by kratom leaf extract against *Cutibacterium acnes*. The extract was tested at 1%, 0.5%, 0.25%, and 0.125% (w/v). Antibacterial activity was assessed by disc diffusion, whereas biofilm formation inhibition was quantified by crystal violet staining in 96-well microtiter plates with optical density measurement at 595 nm, following established antibacterial and biofilm assay approaches (Nasution *et al.*, 2023; Tobi *et al.*, 2022; Hamzah *et al.*, 2021).

Materials

The materials used in this study included 2 N sulfuric acid, concentrated hydrochloric acid, distilled water, Brain Heart Infusion (BHI) medium, Nutrient Agar (NA), 96% ethanol, 1% FeCl₃, chloroform, magnesium powder, Lugol's solution, 0.05 M ammoniacal chloroform, crystal violet, Mayer's reagent, Liebermann-Burchard reagent, and safranin. Medi-Klin® topical solution containing clindamycin phosphate 1% (w/v) was used as the positive control in the antibacterial and biofilm formation inhibition assays.

Methods

Plant material

Fresh mature leaves of *Mitragyna speciosa* Korth. were collected from a single plant in Senyur Village, Muara Ancalong District, East Kutai Regency, East Kalimantan Province, Indonesia.

Plant authentication

Botanical identification and authentication were performed at the Laboratory of Ecology and Tropical Forest Biodiversity Conservation, Faculty of Forestry, Mulawarman University, Samarinda, Indonesia, using morphological characteristics and taxonomic references. The determination certificate number was 298/UN17.4.08/LL/2023.

Preparation of extract

Fresh mature kratom leaves were air-dried at room temperature and ground into powder. A total of 200 g of the powdered material was placed in a dark container and macerated with 96% ethanol for 72 h, with stirring every 8 h and protection from direct sunlight. After maceration, the mixture was allowed to stand overnight and subsequently filtered to separate the plant residue from the macerate. The resulting filtrate was concentrated using a rotary evaporator at 40°C and further concentrated in a water bath until a thick ethanolic extract was obtained. The extraction procedure was based on maceration methods described for ethanolic plant extracts (Hamzah *et al.*, 2021; Asworo & Widhiastuti, 2023).

Qualitative phytochemical screening

The ethanolic kratom leaf extract was qualitatively screened for alkaloids, flavonoids, terpenoids/steroids, phenolic compounds, and saponins using standard color and precipitation reactions (Octaviani *et al.*, 2022; Masriani *et al.*, 2023). These tests were used only to indicate the presence or absence of broad metabolite classes and were not a chemical standardization assay. No specific positive reference controls were used for the qualitative phytochemical reactions.

Alkaloid test

Kratom leaf extract was mixed with 10 mL of ammoniacal chloroform and 2 N sulfuric acid. The mixture was shaken for 2 min and allowed to separate into two layers. The upper layer was collected and treated with Mayer reagent. Formation of a yellowish-white precipitate was interpreted as a positive reaction for alkaloids (Siskawati *et al.*, 2023).

Flavonoid test

Kratom leaf extract was treated with 0.2 g magnesium powder and several drops of concentrated HCl. Development of an orange-red to purple color was interpreted as a positive reaction for flavonoids (Kurnianto *et al.*, 2021).

Terpenoid and steroid test

Kratom leaf extract was treated with two drops of Liebermann-Burchard reagent. A brownish-red/red-purple color was interpreted as a positive reaction for terpenoids, whereas a blue-green

color was interpreted as a positive reaction for steroids (Irfansyah *et al.*, 2024).

Phenolic test

Kratom leaf extract was treated with 1% FeCl₃. Development of a dark green to black coloration was interpreted as a positive reaction for phenolic compounds (Masriani *et al.*, 2023; Bawekes *et al.*, 2023).

Saponin test

Kratom leaf extract was mixed with 10 mL distilled water and shaken vigorously for 10 min. Persistent foam of approximately 1–10 cm that remained for 15 min was interpreted as a positive reaction for saponins (Masriani *et al.*, 2023).

Bacterial strain, culture conditions, and inoculum preparation

Cutibacterium acnes ATCC 6919 was used as the test microorganism. The bacterium was cultured in Brain Heart Infusion (BHI) medium at 37°C for 24 h. The bacterial suspension was adjusted spectrophotometrically to an optical density of 0.1 at 600 nm (OD₆₀₀ = 0.1) and subsequently diluted to OD₆₀₀ = 0.01 for use in the antibacterial and biofilm formation inhibition assays, following the inoculum-preparation approach reported by Mochtar *et al.* (2022). No strain-specific OD-to-CFU calibration or viable-count verification was documented; therefore, the inoculum density is reported based on OD₆₀₀ rather than as an estimated CFU/mL. The source of the working culture and the atmospheric conditions used during cultivation and subsequent assays were not documented in the available study records.

Gram staining

A bacterial smear was prepared on a microscope slide, air-dried, and sequentially stained with crystal violet, Lugol iodine, decolorized with alcohol, and counterstained with safranin according to a standard Gram-staining procedure (Deswita *et al.*, 2021). Stained preparations were examined microscopically to confirm the Gram-positive coryneform morphology expected for *C. acnes* (Pribadhi *et al.*, 2023). Cell dimensions were not used as a quantitative identification criterion in this study.

Antibacterial activity

Antibacterial activity was evaluated using the disc diffusion method on Nutrient Agar (NA) inoculated with a standardized suspension of *Cutibacterium acnes* ATCC 6919. Kratom leaf extract was prepared at concentrations of 1%, 0.5%, 0.25%, and 0.125% (w/v), and sterile paper discs were immersed in the respective extract solutions before being placed on the inoculated agar surface. Medi-Klin® solution containing 1% (w/v) clindamycin phosphate was used as the positive control and was applied using the same disc-immersion procedure. A negative control was included for comparison. The plates were incubated at 37°C for 24 h. After incubation, the diameters of the clear inhibition zones surrounding the discs were measured in millimeters in two perpendicular directions. Each treatment was evaluated in triplicate (n = 3), and the results were expressed as mean ± standard deviation. The disc-diffusion procedure was adapted from established antibacterial testing of ethanolic plant extracts (Nasution *et al.*, 2023).

Biofilm formation inhibition assay (24 and 48 h)

The assay was designed to evaluate inhibition of biofilm formation rather than eradication of preformed biofilms. Each treatment well

contained 75 µL of Brain Heart Infusion (BHI) medium, 15 µL of *Cutibacterium acnes* ATCC 6919 suspension, and 10 µL of kratom leaf extract at concentrations of 1%, 0.5%, 0.25%, or 0.125% (w/v), resulting in a final volume of 100 µL per well. BHI medium without bacterial inoculum was used as the blank, whereas bacterial suspension without extract treatment served as the untreated growth control (negative control). Medi-Klin® solution containing 1% (w/v) clindamycin phosphate was used as the positive control. The microplates were incubated at 37°C for 24 or 48 h. After incubation, the wells were washed three times with sterile distilled water to remove non-adherent cells. Subsequently, 125 µL of 1% crystal violet solution was added to each well to stain the attached biofilm biomass. The wells were then washed three times to remove excess crystal violet, and the retained stain was solubilized with 200 µL of 96% ethanol. Optical density was measured at 595 nm using a microplate reader. Each treatment and control was evaluated in triplicate (n = 3). The crystal violet microtiter-plate procedure was based on previously reported biofilm assays (Tobi *et al.*, 2022; Hamzah *et al.*, 2021; Henra *et al.*, 2025; Henra *et al.*, 2026). The percentage of biofilm formation inhibition was calculated using the following equation:

$$\text{Biofilm Inhibition (\%)} = \frac{(\text{OD}_{\text{untreated control}} - \text{OD}_{\text{treated sample}})}{\text{OD}_{\text{untreated control}}} \times 100$$

Where OD untreated control represents the mean OD595 of the untreated bacterial growth control, and OD treated sample represents the mean OD595 of the corresponding extract-treated group. The lowest tested concentration producing ≥ 50% inhibition of biofilm formation was identified for each incubation period.

Statistical analysis

Three replicate measurements were available for each experimental group (n = 3). Data are presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. For the antibacterial assay, differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant

difference (HSD) post hoc test. For the biofilm formation inhibition assay, one-way ANOVA was performed separately for the 24-h and 48-h incubation periods using the four extract-treatment groups and the positive-control group. Tukey's HSD post hoc test was applied when the overall ANOVA indicated a statistically significant difference. Statistical significance was set at $p < 0.05$. The untreated bacterial growth control was used as the reference for calculating the percentage of biofilm formation inhibition and was not included among the five treatment groups used for post hoc comparisons. Statistical analyses were performed using GraphPad Prism 8.0.

RESULTS AND DISCUSSION

The extract

Maceration of 200 g of dried kratom leaf powder with 96% ethanol yielded 38.96 g of concentrated extract, corresponding to a yield of

19.48% (Table 1). The use of 96% ethanol is consistent with the extraction procedure and extract-yield data reported for this study. Extraction yield is influenced by solvent composition, extraction time, particle characteristics, and other processing conditions (Asworo & Widwastuti, 2023; Senduk *et al.*, 2020).

Table 1. Results of extract yield

Solvent	Weight of Simplisia (g)	Extract Weight (g)	Yield (%)
Ethanol 96%	200	38.96	19.48

The 19.48% yield reflects the mass of concentrated extract recovered relative to the starting dried plant material. Maceration is a low-temperature extraction procedure commonly used for plant materials because it can limit thermal degradation of heat-sensitive constituents (Asworo & Widwastuti, 2023). Because extraction yield depends on solvent composition, particle size, plant-to-solvent ratio, extraction time, and residual moisture, direct comparison with other studies should be made only when these parameters are comparable (Senduk *et al.*, 2020). The present yield therefore describes extraction recovery and should not be interpreted as a direct measure of biological potency.

The use of 96% ethanol allows extraction of a broad range of relatively polar and moderately non-polar constituents and is commonly used in phytochemical studies of plant materials (Wendersteyt *et al.*, 2021; Yuniarti *et al.*, 2020). However, the extract was not chemically standardized in the present study; consequently, the concentration of mitragynine or other individual constituents in the test solutions is unknown.

After solvent removal at 40°C, the concentrated extract was used for qualitative phytochemical screening and preparation of the antibacterial and biofilm-formation test concentrations. Similar low-temperature concentration approaches have been used for ethanolic plant extracts to obtain concentrated material for subsequent biological testing (Hamzah *et al.*, 2021).

Screening of phytochemicals

Qualitative phytochemical screening detected alkaloids, flavonoids, steroids, and phenolic compounds, whereas terpenoids and saponins were not detected (Table 2). These findings are broadly consistent with previous reports showing that kratom leaves contain multiple classes of secondary metabolites, although the detected profile may vary with plant source and extraction conditions (Yuniarti *et al.*, 2020; Masriani *et al.*, 2023; Maulia *et al.*, 2021).

The screening results provide only class-level qualitative information. Differences between studies may arise from plant origin, solvent composition, extraction conditions, and the sensitivity of color- or precipitation-based reactions (Putri & Lubis, 2020; Masriani *et al.*, 2023). In the present study, no quantitative alkaloid, flavonoid, or phenolic assay and no chromatographic profiling were performed. Therefore, these results cannot identify the compound or compound class responsible for the antibacterial or biofilm formation inhibitory activity.

The positive Mayer reaction supported the presence of alkaloids, consistent with the formation of a characteristic precipitate in qualitative alkaloid testing (Putri & Lubis, 2020). The orange color in the magnesium/HCl reaction supported the presence of flavonoids (Dewi *et al.*, 2021). The green Liebermann-Burchard reaction was consistent with steroids, whereas the absence of the characteristic terpenoid coloration supported a negative terpenoid result (Nurjannah *et al.*, 2022). The

FeCl₃ reaction supported the presence of phenolic compounds (Bawekes *et al.*, 2023). The saponin reaction was negative because persistent foam was not observed. These observations

should be interpreted as preliminary phytochemical screening rather than chemical standardization of the extract.

Table 2. Results of phytochemical screening.

Compound	Result	Description
Alkaloid	+	White precipitate
Flavonoid	+	Orange color
Terpenoid	-	No red/purple coloration
Steroid	+	Green coloration
Phenolic	+	Dark green/black coloration
Saponin	-	Foam not persistent

Description:

(-) Negative = Does not contain compounds; (+) Positive = Contains compounds

Identification of *Cutibacterium acnes*

Microscopic examination after Gram staining showed purple, pleomorphic rod-shaped/coryneform cells consistent with a Gram-positive *Cutibacterium* morphology (Figure 1), in agreement with previous descriptions of *C. acnes* morphology and Gram-staining characteristics (Sasebohe *et al.*, 2023; Pribadhi *et al.*, 2023). Gram staining was used as a basic morphological quality check. The test strain used in this study was *C. acnes* ATCC 6919, as specified in the Materials and Methods.

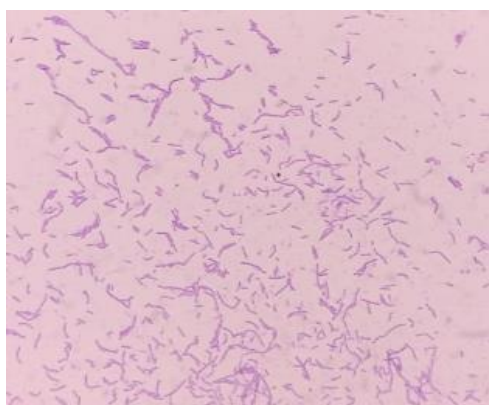


Figure 1. *Cutibacterium acnes* ATCC 6919 after Gram staining.

Antibacterial results

Antibacterial testing using the disc diffusion method produced clear inhibition zones around the discs. Figure 2 shows the inhibition zones

obtained at the tested extract concentrations and with the positive control.

Among the tested extract concentrations, 1% (w/v) produced the largest mean inhibition zone (10.83 ± 0.76 mm), followed by 0.5% (8.67 ± 2.08 mm), 0.25% (5.50 ± 1.00 mm), and 0.125% (3.67 ± 1.26 mm). The positive control (clindamycin phosphate 1% w/v) produced an inhibition zone of 11.67 ± 3.06 mm, whereas the negative control produced no measurable inhibition zone. One-way ANOVA showed an overall difference among the five treatment groups, $F(4,10) = 10.45$, $p = 0.0013$. Tukey's HSD showed significant differences between 0.125% and 0.5% (adjusted $p = 0.0465$), 0.125% and 1% ($p = 0.0052$), 0.125% and the positive control ($p = 0.0024$), 0.25% and 1% ($p = 0.0329$), and 0.25% and the positive control ($p = 0.0140$); all other pairwise comparisons were not significant. These results indicate a concentration-related increase in inhibition-zone diameter. Descriptive categories such as “strong,” “moderate,” or “weak” were not used because no validated interpretive standard for crude plant extracts was applied. Disc-diffusion zone diameters reflect both antibacterial activity and diffusion of extract constituents through agar and should not be interpreted using clinical antibiotic breakpoints. Previous studies likewise reported antibacterial activity of kratom preparations against acne-associated bacteria, although direct comparison is limited by differences in plant material, extraction procedure, test concentration, and assay

conditions (Suhaimi *et al.*, 2019; Putri *et al.*, 2023). Clindamycin, used here as the positive control, is a lincosamide antibiotic that inhibits

bacterial protein synthesis through binding to the 50S ribosomal subunit (Luchian *et al.*, 2021).

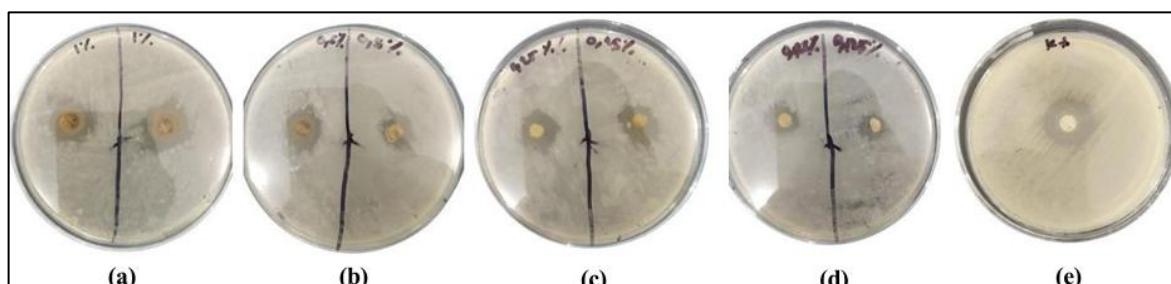


Figure 2. Disc-diffusion assay of kratom leaf extract at (a) 1%, (b) 0.5%, (c) 0.25%, and (d) 0.125% (w/v), and (e) positive control (Medi-Klin, clindamycin phosphate 1% w/v solution).

Biofilm formation inhibition results

Crystal violet measurements were converted to percentage inhibition relative to the untreated growth control. Because the extract was present from the beginning of incubation, the assay quantifies prevention/inhibition of biofilm formation rather than eradication of an established biofilm, consistent with the interpretation of microtiter-plate biofilm inhibition assays (Tobi *et al.*, 2022; Hamzah *et al.*, 2021). The mean inhibition values at 24 and 48 h are summarized in Figure 3.

At 24 h, the positive control produced 79.18% biofilm inhibition, while kratom extract produced 70.67%, 65.84%, 49.93%, and 46.44% inhibition at 1%, 0.5%, 0.25%, and 0.125% (w/v), respectively. The one-way ANOVA across the positive-control and extract-treatment groups was significant, $F(4,10) = 5.50$, $p = 0.0133$. Tukey's HSD identified significant differences between the positive control and 0.25% extract (adjusted $p = 0.0367$) and between the positive control and 0.125% extract ($p = 0.0193$); all other pairwise comparisons were not significant. At 48 h, the positive control produced 65.91% inhibition, and the extract produced 58.75%, 56.94%, 45.61%, and 40.53%

inhibition at 1%, 0.5%, 0.25%, and 0.125% (w/v), respectively. The overall difference among these five groups was not significant, $F(4,10) = 2.11$, $p = 0.1537$. At both time points, 0.5% (w/v) was the lowest tested extract concentration producing at least 50% inhibition. Because the tested concentrations were discrete and no validated dose-response model was fitted, 0.5% is reported as the lowest tested concentration meeting the $\geq 50\%$ criterion rather than as an exact MBIC₅₀. The lower inhibition observed after 48 h than after 24 h is consistent with the greater persistence and structural complexity that can develop as *C. acnes* biofilms mature, although the present assay does not directly measure matrix architecture or biofilm viability (Coenye *et al.*, 2022; Henra, *et al.*, 2025; Henra *et al.*, 2026). Studies of plant-derived compounds against *C. acnes* have similarly shown that inhibition of biofilm formation can occur independently of eradication of established biofilms (De Canha *et al.*, 2020). The observed concentration-response trend may reflect greater exposure to one or more extract constituents at higher concentrations; however, the qualitative phytochemical data do not identify the active compound(s), and specific attribution to alkaloids or mitragynine is not supported by this study.

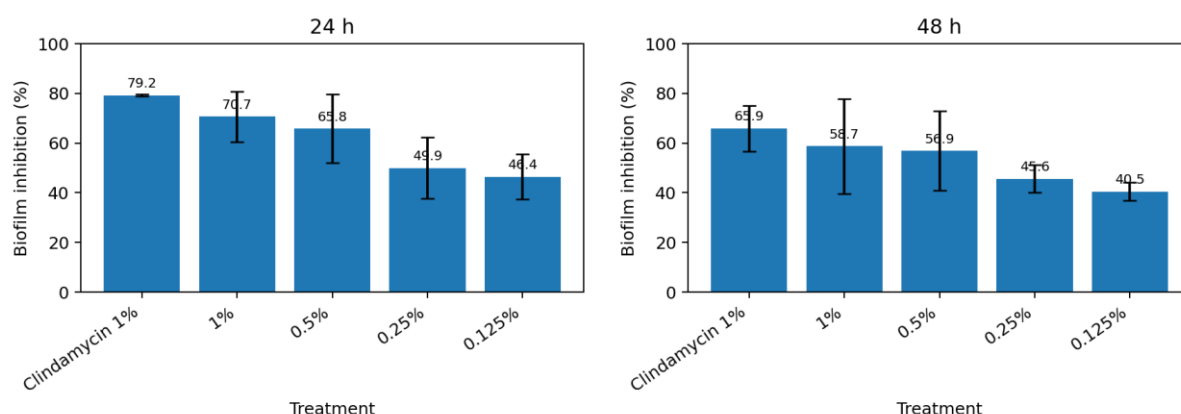


Figure 3. Inhibition of *Cutibacterium acnes* biofilm formation after 24 h and 48 h of incubation. Bars represent mean $\pm$ SD derived from three replicate measurements ($n = 3$) reported in the thesis. Percent inhibition was calculated relative to the untreated growth control. At 24 h, Tukey's HSD indicated no significant difference between clindamycin phosphate 1% and the 1% kratom extract (adjusted $p = 0.8427$). At 48 h, the overall ANOVA was not significant; therefore, post hoc significance claims were not made.

CONCLUSIONS

The 96% ethanolic leaf extract of kratom (*Mitragyna speciosa* Korth.) showed antibacterial activity and inhibited *Cutibacterium acnes* ATCC 6919 biofilm formation under the conditions tested. The 1% (w/v) extract produced the largest antibacterial inhibition zone among the extract treatments (10.83 ± 0.76 mm) and the highest biofilm inhibition among the extract concentrations, reaching 70.67% at 24 h and 58.75% at 48 h. The lowest tested concentration producing $\geq 50\%$ biofilm inhibition was 0.5% (w/v) at both time points; an exact MBIC₅₀ was not determined because a validated dose-response model was not fitted. Qualitative phytochemical screening detected alkaloids, flavonoids, steroids, and phenolic compounds; however, the specific compounds responsible for the antibacterial and biofilm formation inhibitory activities remain unknown. Further studies should chemically standardize the extract, identify active constituents, validate activity across well-characterized *C. acnes* strains, and clarify mechanisms of action.

AUTHOR CONTRIBUTIONS (CRediT)

Ramayta Azizah, Henra Henra, Hasyrul Hamzah, Nur Atika Astriani, Sajida Khairallah Divatunnisa, Nita Talia and Suhardiansyah: Investigation, Methodology, Formal Analysis, Writing – Original Draft, and Writing – Review & Editing. This role statement reflects the

contribution description provided in the submitted manuscript and should be confirmed by all authors before resubmission.

FUNDING

This study received financial support from Universitas Muhammadiyah Kalimantan Timur and the RisetMu Research Funding Program, Indonesia.

COMPETING INTERESTS

The authors declare that they have no competing interests related to this study.

REFERENCES

- Asworo, R. Y., & Widwastuti, H. (2023). Pengaruh ukuran serbuk simplisia dan waktu maserasi terhadap aktivitas antioksidan ekstrak kulit sirsak. *Indonesian Journal of Pharmaceutical Education*, 3(2), 256–263. <https://doi.org/10.37311/ijpe.v3i2.19906>
- Bawekes, S. M., Yudistira, A., & Rumondor, E. M. (2023). Uji kualitatif kandungan senyawa kimia perasan jeruk nipis (*Citrus aurantifolia* Swingle). *Pharmakon*, 12(3), 373–377. <https://doi.org/10.35799/pha.12.2023.49269>
- Coenye, T., Spittaels, K.-J., & Achermann, Y. (2022). The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of *Cutibacterium acnes*. *Biofilm*, 4, 100063. <https://doi.org/10.1016/j.biofilm.2021.100063>
- De Canha, M. N., Komarnytsky, S., Langhansova, L., & Lall, N. (2020). Exploring the anti-acne

- potential of Impepho *Helichrysum odoratissimum* (L.) Sweet to combat *Cutibacterium acnes* virulence. *Frontiers in Pharmacology*, 10, 1559. <https://doi.org/10.3389/fphar.2019.01559>
- Deswita, W., Manalu, K., & Tambunan, E. P. S. (2021). Uji efektivitas antibakteri ekstrak umbi lobak putih (*Raphanus sativus* L.) terhadap pertumbuhan bakteri *Propionibacterium acnes* dan *Staphylococcus epidermidis*. *Klorofil: Jurnal Ilmu Biologi dan Terapan*, 5(2), 111. <https://doi.org/10.30821/kfl:jibt.v5i2.10032>
- Dewi, I. S., Saptawati, T., & Rachma, F. A. (2021). Skrining fitokimia ekstrak etanol kulit dan biji terong belanda (*Solanum betaceum* Cav.). *Prosiding Seminar Nasional UNIMUS*, 1210–1218.
- Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A., & Roques, C. (2018). *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: A brief look at the latest updates. *Journal of the European Academy of Dermatology and Venereology*, 32(2), 5–14. <https://doi.org/10.1111/jdv.15043>
- Firmansyah, A., Sundalian, M., & Taufiq, M. (2021). Kratom (*Mitragyna speciosa* Korth) for a new medicinal: A review of pharmacological and compound analysis. *Biointerface Research in Applied Chemistry*, 11(2), 9704–9718. <https://doi.org/10.33263/BRIAC112.97049718>
- Hamzah, H., Rasdianah, N., Nurwijayanto, A., & Nandini, E. (2021). Aktivitas ekstrak etanol daun calincing terhadap biofilm *Candida albicans*. *Jurnal Farmasetis*, 10(1), 21–28. <https://doi.org/10.32583/farmasetis.v10i1.1319>
- Henra, H., Damayanti, E., & Widada, J. (2025). Antifungal and antibiofilm activity of *Bacillus velezensis* BP1 extract against *Candida albicans*: In vitro bioassay, metabolomics, in silico molecular docking. *Trends in Sciences*, 22, 9971. <https://doi.org/10.48048/tis.2025.9971>
- Henra, H., Damayanti, E., & Widada, J. (2026) Potent Anti-Biofilm Action of *Bacillus* BPY1 and BPY2 Extracts against *Candida albicans* revealed by Metabolomics. *Research Journal of Pharmacy and Technology*. 19(8):3523-3530. doi: 10.52711/0974-360X.2026.00499
- Irfansyah, F. D., Fatimah, & Junairiah. (2024). Skrining fitokimia dan aktivitas antioksidan tiga jenis tabebuia (*Tabebuia* spp.). *Berita Biologi*, 23(1), 49–59. <https://doi.org/10.55981/beritabiologi.2024.1668>
- Kurnianto, E., Rahman, I. R., & Hairunnisa. (2021). Skrining fitokimia ekstrak etanol daun matoa yang berasal dari Pontianak Timur dengan variasi konsentrasi pelarut. *Jurnal Komunitas Farmasi Nasional*, 1(2), 131–138.
- Luchian, I., Goriuc, A., Martu, M. A., & Covasa, M. (2021). Clindamycin as an alternative option in optimizing periodontal therapy. *Antibiotics*, 10(7), 814. <https://doi.org/10.3390/antibiotics10070814>
- Masriani, M., Muharini, R., Wijayanti, D. K., Melanie, P., & Widiarsari, M. L. (2023). Phytochemical screening of ethanol extracts from three variants of kratom leaves (*Mitragyna speciosa* Korth.). *Hydrogen: Jurnal Kependidikan Kimia*, 11(2), 192. <https://doi.org/10.33394/hjkk.v11i2.7122>
- Maulia, S. W., Jubaidah, S., & Siswanto, E. (2021). Uji toksisitas akut ekstrak etanol daun kratom (*Mitragyna speciosa* Korth.) dengan metode maserasi dan refluks terhadap larva *Artemia salina* Leach. *Prosiding Seminar Nasional Kesehatan*, 1, 75–85.
- Mochtar, C. F., Saleh, L. O., Hamzah, H., & Ilyas, N. M. (2022). Potensi bajakah tampala (*Spatholobus littoralis* Hassk) sebagai antibakteri dan antijamur terhadap *Staphylococcus aureus* dan *Candida albicans*. *Jurnal Mandala Pharmacon Indonesia*, 8(2), 177–184. <https://doi.org/10.35311/jmpi.v8i2.212>
- Nasution, M. A., Andry, M., Syahputri, H., Pertiwi, N. N., & Sari, M. (2023). Uji aktivitas antibakteri ekstrak etanol daun sisik naga terhadap bakteri *Staphylococcus aureus* dan *Salmonella typhi*. *Jurnal Dunia Farmasi*, 7(2), 125–136. <https://doi.org/10.33085/jdf.v7i2.5693>
- Nurjannah, I., Mustariani, B. A. A., & Suryani, N. (2022). Skrining fitokimia dan uji antibakteri ekstrak kombinasi daun jeruk purut (*Citrus hystrix*) dan kelor (*Moringa oleifera* L.) sebagai zat aktif pada sabun antibakteri. *SPIN*, 4(1), 23–36. <https://doi.org/10.20414/spin.v4i1.4801>
- Octaviani, M., Alfitri, N., & Fadhli, H. (2022). Antibacterial activity of fraction of *Allium cepa* L. tubers. *Indonesian Journal of Pharmaceutical Science and Technology*, 9(1), 56. <https://doi.org/10.24198/ijpst.v1i1.29474>
- Pribadhi, A. N., Mastuti, S., & Purwaningrum, E. (2023). Aktivitas antibakteri dari bakteri probiotik dalam melawan *Propionibacterium acnes* dan *Staphylococcus epidermidis*. *Indobiosains*, 5(1), 1–7. <https://doi.org/10.31851/indobiosains.v5i1.9659>
- Puspasari, H., & Krismonika, I. F. (2020). Uji daya hambat ekstrak kental daun kratom (*Mitragyna speciosa* Korth) terhadap pertumbuhan bakteri *Staphylococcus epidermidis* sebagai penyebab jerawat. *Medika Sains: Jurnal Ilmiah*

- Kefarmasian*, 4(2), 87–94.
<https://doi.org/10.37874/ms.v4i2.126>
- Putri, D. M., & Lubis, S. S. (2020). Skrining fitokimia ekstrak etil asetat daun kalayu (*Erioglossum rubiginosum* (Roxb.) Blum). *Amina*, 2(3), 120–125.
- Putri, M., Wardoyo, E. R. P., & Kurniatuhadi, R. (2023). Potensi ekstrak metanol akar dan batang kratom (*Mitragyna speciosa* Korth.) sebagai antibakteri *Propionibacterium acnes* ATCC 6919 penyebab jerawat. *Protobiont*, 12(2), 43–49.
<https://doi.org/10.26418/protobiont.v12i2.70835>
- Sasebohe, V. Y., Prakasita, V. C., & Aditiyarini, D. (2023). Aktivitas antibakteri ekstrak etanol daun binahong terhadap *Staphylococcus aureus* dan *Propionibacterium acnes* penyebab jerawat. *Sciscitatio*, 4(1).
<https://doi.org/10.21460/sciscitatio.2023.41.107>
- Senduk, T. W., Montolalu, L. A. D. Y., & Dotulong, V. (2020). Rendemen ekstrak air rebusan daun tua mangrove *Sonneratia alba*. *Jurnal Perikanan dan Kelautan Tropis*, 11(1), 9.
<https://doi.org/10.35800/jpkt.11.1.2020.28659>
- Siskawati, Haeruddin, & Nurlansi. (2023). Uji fitokimia dan aktivitas antioksidan ekstrak metanol daun kelor (*Moringa oleifera*). *Jurnal Kimia dan Pendidikan Kimia*, 12(1), 1–9.
- Sudarwati, T. P. L. (2021). Uji antimikroba fraksi III daun kratom (*Mitragyna speciosa*) terhadap bakteri *Staphylococcus aureus*. *Jurnal Komunitas Farmasi Nasional*, 1(2), 6.
- Suhaimi, Puspasari, H., Husnani, & Apriani, M. (2019). Aktivitas antibakteri ekstrak daun kratom (*Mitragyna speciosa* Korth.) terhadap bakteri *Propionibacterium acnes* sebagai penyebab jerawat. *Medika Sains: Jurnal Ilmiah Kefarmasian*, 4(1), 1–6.
- Tobi, C. H. B., Saptarini, O., & Rahmawati, I. (2022). Aktivitas antibiofilm ekstrak dan fraksi-fraksi biji pinang (*Areca catechu* L.) terhadap *Staphylococcus aureus* ATCC 25923. *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, 7(1), 56.
<https://doi.org/10.20961/jpscr.v7i1.43698>
- Wendersteyt, N. V., Wewengkang, D. S., & Abdullah, S. S. (2021). Uji aktivitas antimikroba dari ekstrak dan fraksi ascidian *Herdmania momus* dari perairan Pulau Bangka Likupang terhadap pertumbuhan mikroba *Staphylococcus aureus*, *Salmonella typhimurium* dan *Candida albicans*. *Pharmacon*, 10(1), 706.
<https://doi.org/10.35799/pha.10.2021.32758>
- Yuniarti, R., Nadia, S., Alamanda, A., Zubir, M., Syahputra, R. A., & Nizam, M. (2020). Characterization, phytochemical screenings and antioxidant activity test of kratom leaf ethanol extract (*Mitragyna speciosa* Korth) using DPPH method. *Journal of Physics: Conference Series*, 1462(1), 012026. <https://doi.org/10.1088/1742-6596/1462/1/012026>