

Antibacterial Activity of Emulgel Containing Japanese Papaya Leaf (*Cnidoscolus aconitifolius*) Extract Against *Staphylococcus epidermidis* and *Propionibacterium acnes*

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ABSTRACT

Acne is a common skin disorder associated with the presence of *Staphylococcus epidermidis* and *Propionibacterium acnes*. Excessive use of antibiotics may increase the risk of resistance, highlighting the need to develop antibacterial agents from natural sources. Japanese papaya (*Cnidoscolus aconitifolius*) leaves have been reported to contain secondary metabolites with potential antibacterial activity. This study aimed to determine the antibacterial activity of Japanese papaya leaf extract emulgel against *Staphylococcus epidermidis* and *Propionibacterium acnes* and to determine the extract concentration that exhibited the highest antibacterial activity. An experimental in vitro study was conducted using the well diffusion method, with extraction performed by maceration using 70% ethanol. The emulgel was formulated at three extract concentrations, namely 10%, 15%, and 20%, with clindamycin as the positive control and emulgel base as the negative control. Physical evaluation showed that Formula 3 (20%) was less homogeneous due to the presence of a small amount of particles, while several preparations exhibited spreadability below the 5–7 cm range. The pH values of Formula 1, Formula 2, Formula 3, and the positive control were within the range of 4.5–8.0, whereas the negative control had a pH of 4.42. The Japanese papaya (*Cnidoscolus aconitifolius*) leaf extract emulgel demonstrated antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes*, although the observed activity varied depending on the bacterial species and extract concentration. The 15% formulation was identified as the optimal concentration based on the study findings. However, its relative effectiveness compared with the positive and negative controls should be explicitly stated using the corresponding inhibition-zone measurements. The positive control should be used to assess the formulation's activity relative to the reference antibacterial agent, while the negative control should be used to determine whether the emulgel base itself contributes to bacterial growth inhibition.

Keywords: emulgel, Japanese papaya leaf, antibacterial, *Staphylococcus epidermidis*, *Propionibacterium acnes*

INTRODUCTION

Bacterial skin infections remain a major health problem, particularly in developing countries, including Indonesia. *Propionibacterium acnes* plays an important role in the pathogenesis of acne vulgaris, a chronic inflammatory disease of the pilosebaceous unit (Saragih *et al.*, 2016). Acne vulgaris affects 80–85% of adolescents aged 15–18 years, with peak prevalence among young adults at 16–19 years in males and 14–17 years in females (Sibero *et al.*, 2019). In addition to *P. acnes*, *Staphylococcus epidermidis* is also associated with acne and skin inflammation.



Acne is treated with topical and systemic therapies, including antibiotics such as clindamycin, tetracycline, erythromycin, cephalosporins, sulfonamides, and trimethoprim (Desinta *et al.*, 2024). However, prolonged antibiotic use may increase antimicrobial resistance and reduce therapeutic effectiveness (Ardiyanto Saidin Marsudi *et al.*, 2021). Therefore, natural antibacterial agents are needed as potential alternatives for topical preparations (Desinta *et al.*, 2024).

Japanese papaya (*Cnidoscolus aconitifolius*) leaves contain secondary metabolites, including flavonoids, alkaloids, saponins, and tannins, which may contribute to antibacterial activity (Maria Fatmadewi Imawati *et al.*, 2023). Previous research reported that 30% Japanese papaya leaf extract produced the largest inhibition zone (17,296 mm) compared with 10% and 20% concentrations, indicating its antibacterial potential (Maria Fatmadewi Imawati *et al.*, 2023).

Japanese papaya leaf extract can be formulated as an emulgel to facilitate topical application and contact between the active compounds and the skin. Emulgel combines the properties of emulsions and gels, making it a suitable delivery system for topical preparations (Yasmin *et al.*, 2017). Compared with conventional gels, emulgels can facilitate the incorporation of active compounds with different solubility characteristics, including lipophilic compounds. In addition, their gel base may provide suitable consistency, good spreadability, and ease of application. These properties may be beneficial for acne-prone skin because the formulation can be applied directly to the affected area. However, the effectiveness of an emulsifier depends on its composition and requires experimental evaluation; its superiority over other topical dosage forms should not be assumed without direct comparative evidence.

Therefore, this study aimed to evaluate the antibacterial activity of Japanese papaya (*Cnidoscolus aconitifolius*) leaf against extract formulated as an emulgel against *Staphylococcus epidermidis* and *Propionibacterium acnes* , and to determine the extract concentration that produced the optimal inhibition zone against each bacterial species.

METHODS

Research Design

This study employed an in vitro experimental design to evaluate the antibacterial activity of Japanese papaya (*Cnidoscolus aconitifolius*) leaf extract emulgel against *Staphylococcus epidermidis* and *Propionibacterium acnes* . Three formulations containing 10%, 15%, and 20% extract were tested using the agar well diffusion method, with emulgel base and 1% clindamycin gel as negative and positive controls, respectively. Each treatment was tested in triplicate, and antibacterial activity was assessed by measuring inhibition-zone diameters in millimeters.

Materials and Research Location

Fresh Japanese papaya leaves were collected in January 2026 from Tosaren District, Kediri City, East Java, Indonesia, using purposive sampling. Plant identification, simplicia preparation, and extraction were conducted at UPT Laboratories Materia Medica, Batu, Malang, while emulgel preparation and antibacterial testing were performed at STRADA Indonesia University, Kediri, from January to March 2026. The materials included 70% ethanol, distilled water, Nutrient Agar (NA), Nutrient Broth (NB), bacterial cultures of *S. epidermidis* and *P. acnes* , 1% clindamycin gel, carbomer, liquid paraffin, Tween 80, methylparaben, propylparaben, propylene glycol, and phytochemical screening reagents. Equipment included an analytical balance, grinder, rotary evaporator, water bath, magnetic stirrer, autoclave, incubator, micropipette, Petri dishes, cork borer, vortex mixer, and caliper.

Plant Processing, Extraction, and Phytochemical Screening

Approximately 10 kg of leaves were sorted, washed, cut, dried at 40–60°C, ground, and sieved to obtain simplicia powder. The powder was macerated in 70% ethanol at a ratio of 1:4 for three days in a closed container protected from light, with agitation twice daily. The filtrate was concentrated using a rotary evaporator at 60°C and further thickened in a water bath at 60°C. The extract yield was calculated as the percentage ratio of concentrated extract mass to dried powder mass. Phytochemical screening was conducted for alkaloids using Wagner's and Dragendorff's reagents, flavonoids using magnesium powder and concentrated hydrochloric acid, saponins using the foam test, and tannins using an appropriate detection reagent. Positive results were indicated by characteristic precipitates, color changes, or persistent foam, as appropriate.

Emulgel Preparation and Evaluation

Carbomer was dispersed in distilled water and allowed to hydrate to form the gel base. Liquid paraffin constituted the oil phase, while Tween 80, methylparaben, propylparaben, and propylene glycol were incorporated into the aqueous phase. Both phases were heated separately to 70–80°C, after which the oil phase was added to the aqueous phase while stirring at 300 rpm. The resulting emulsion was incorporated into the gel base and mixed until uniform. After cooling to approximately 28–30°C, the extract was added to produce 10%, 15%, and 20% formulations. The formulations were evaluated for organoleptic characteristics, homogeneity, pH, and spreadability. An ethanol-free test was also performed according to the study protocol. The complete composition of each formulation, including the quantities of all excipients, should be reported in a formulation table..

Antibacterial Activity Test

Equipment and suitable culture media were sterilized by autoclaving at 121°C for 15 minutes. NA and NB were prepared according to the respective manufacturers' instructions. Bacterial cultures were rejuvenated in NB at 37°C for 24 hours, and suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard. The suspensions of each bacterial species were tested separately using the agar well diffusion method. The treatments consisted of the 10%, 15%, and 20% extract emulgels, the emulgel base as the negative control, and 1% clindamycin gel as the positive control. Each treatment was tested in triplicate, and the plates were incubated at 37°C for 24 hours according to the study protocol. Inhibition zones were measured using a caliper and recorded in millimeters.

Data Analysis

Formulation characteristics were analyzed descriptively, while inhibition-zone data were analyzed using IBM SPSS Statistics version 27. Normality and homogeneity of variance were assessed before inferential testing. One-way ANOVA followed by Tukey's test was used when the assumptions were met; Welch's ANOVA followed by the Games–Howell test was considered for normally distributed data with unequal variances. The Kruskal–Wallis test was used when parametric assumptions were not met. Statistical significance was set at $p < 0.05$.

RESULT

Evaluation of Japanese Papaya Leaf (*Cnidioscolus aconitifolius*) Extract Emulgel
Organoleptic Evaluation

Organoleptic evaluation was performed to observe the physical characteristics of

the preparations, including their form, color, and odor. The observations showed that all formulations were in the form of emulgels, with differences in color and odor among the formulations.



Figure 1. Extract Japanese Papaya Leaf

Table 1. Organoleptic Evaluation Results

Formula	Form	Color	Odor
Formula 1 (10%)	Emulgel	Dark green	Characteristics of the extract
Formula 2 (15%)	Emulgel	Dark green	Characteristics of the extract
Formula 3 (20%)	Emulgel	Dark green	Characteristics of the extract
Negative control	Emulgel	Opaque white	Characteristics of the base
Positive control	Gel	Clear	Odorless

Homogeneity Test Results

The homogeneity test was conducted to determine whether each preparation was uniformly dispersed. A homogeneous preparation is one that does not show any particles when visually observed and is evenly dispersed so that when used in treatment the dosage used can be evenly distributed. The homogeneity test is carried out by placing 0.5 g of the preparation on a glass object and observing visually. The results of the homogeneity test for the Japanese papaya leaf extract emulgel preparation can be seen in Table 2.

Table 2. Results of Homogeneity Test

Formula	Homogeneity	Information
Formula 1 (10%)	There are no coarse grains	Homogeneous
Formula 2 (15%)	There are no coarse grains	Homogeneous
Formula 3 (20%)	There are few particles	Less homogeneous
Control (-)	Homogeneous	Homogeneous
Control (+)	Homogeneous	Homogeneous

Information

Control (-) = No extract content

Control (+) = Clidamycin gel antibiotic (1%)

pH Evaluation

The pH test was performed to determine the acidity of the emulgel preparations. The pH results are presented in Table 3.

Table 3 . pH Evaluation Results of the Emulgel Preparations

Formula	Replication 1	Replication 2	Replication 3	Mean $\pm$ SD
Formula 1 (10%)	5.93	5.97	5.95	5.95 $\pm$ 0.02
Formula 2 (15%)	5.89	5.89	5.84	5.87 $\pm$ 0.03
Formula 3 (20%)	5.92	5.88	5.87	5.89 $\pm$ 0.03
Negative control	4.58	4.34	4.32	4.42 $\pm$ 0.14
Positive control	6.40	6.73	6.81	6.64 $\pm$ 0.22

Note: Standard pH range for topical preparations: 4.5–8.0 (SNI 16-3499-1996).

Spreadability Evaluation

Spreadability was evaluated using loads of 50 and 100 g. The spreadability results of the Japanese papaya leaf extract emulgels are presented in Tables 2 and 3.

Table 4. Spreadability Evaluation of Emulgel Preparations

Formula	50 g (cm)	100 g (cm)
Formula 1 (10%)	4.42 $\pm$ 0.19	4.76 $\pm$ 0.03
Formula 2 (15%)	4.63 $\pm$ 0.06	5.08 $\pm$ 0.05
Formula 3 (20%)	3.78 $\pm$ 0.06	4.07 $\pm$ 0.08
Negative control	4.25 $\pm$ 0.08	5.08 $\pm$ 0.11
Positive control	5.62 $\pm$ 0.05	6.45 $\pm$ 0.05

Note: Spreadability standard: 5–7 cm (Sholaekah, 2025).

Antibacterial Activity against *Staphylococcus epidermidis* and *Propionibacterium acnes*

The antibacterial activity test was performed to determine the ability of Japanese papaya leaf extract emulgel to inhibit the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes*. Antibacterial activity was determined based on the diameter of the inhibition zones formed around the wells. The results are presented in Table 5.

Table 5. The antibacterial activity test

Group	<i>S. epidermidis</i> (mm)	<i>P. acnes</i> (mm)
F1 (10%)	0.00 $\pm$ 0.00	5.45 $\pm$ 0.39
F2 (15%)	6.68 $\pm$ 0.97	6.42 $\pm$ 0.03
F3 (20%)	9.13 $\pm$ 0.96	9.45 $\pm$ 0.40
Negative control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Positive control	29.25 $\pm$ 0.23	26.23 $\pm$ 0.21

Statistical test: ANOVA, $p < 0.001$; Welch ANOVA, $p < 0.001$.

Note: Negative control = emulgel base without extract; positive control = 1% clindamycin gel.

Inhibition zone categories: ≤ 5 mm = weak; 6–10 mm = moderate; 11–20 mm = strong; ≥ 21 mm = very strong (Safitri, 2021).

DISCUSSION

The plant identification confirmed that the sample was Japanese papaya (*Cnidoscopus aconitifolius*). The leaves were extracted using 70% ethanol through maceration for 3 × 24 hours, and the concentrated extract was subsequently used to prepare emulgel formulations. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, and tannins, which may contribute to the extract's antibacterial potential. Total ash content was 10%, within the reported maximum limit of 15.5%. Thin-layer chromatography (TLC) produced R_f values of 0.87, 0.90, 0.87, and 0.91 for alkaloids, flavonoids, saponins, and tannins, respectively; however, these relatively high values should be interpreted cautiously because R_f values alone are insufficient to confirm compound identity.

Physical evaluation showed that all formulations had an emulgel appearance and characteristic extract odor, while the negative control had the characteristic odor of the base. Formulas 1 and 2 were homogeneous, whereas Formula 3 (20% extract) contained visible extract particles, possibly because the higher extract concentration made uniform dispersion more difficult. The pH values of most formulations were within the reference range of 4.5–8.0. Spreadability increased under the higher applied load, from 4.42 to 4.76 cm for Formula 1, 4.63 to 5.08 cm for Formula 2, and 3.78 to 4.07 cm for Formula 3. Formula 2 demonstrated the greatest spreadability among the extract-containing formulations, whereas Formula 3 had the lowest, potentially reflecting differences in consistency and the greater proportion of extract solids. Carbomer contributes to gel structure and viscosity, while liquid paraffin forms the oil phase and Tween 80 facilitates emulsification. Propylene glycol acts as a cosolvent and humectant, while methylparaben and propylparaben function as preservatives. Together, these excipients support formulation stability and topical application, although the effects of individual ingredients were not independently tested.

The antibacterial activity of the emulgels increased with extract concentration, although the response differed between the two bacterial species. Against *Staphylococcus epidermidis*, Formula 1 (10%) produced no measurable inhibition zone, whereas Formula 2 (15%) and Formula 3 (20%) produced mean inhibition zones of 6.68 ± 0.97 mm and 9.13 ± 0.96 mm, respectively. The negative control showed no inhibition, while the 1% clindamycin gel produced a zone of 29.25 ± 0.22 mm. Against *Propionibacterium acnes* (currently classified as *Cutibacterium acnes*), the mean inhibition zones were 5.45 ± 0.39 mm for Formula 1, 6.42 ± 0.03 mm for Formula 2, and 9.45 ± 0.39 mm for Formula 3. The negative control showed no inhibition, while the positive control produced a zone of 26.23 ± 0.20 mm. These findings suggest that Formula 3 had the strongest antibacterial activity among the extract formulations against both bacteria, while clindamycin remained substantially more effective under the conditions tested.

The increase in inhibition-zone diameter with increasing extract concentration may be related to the greater amount of potentially active phytochemicals available to diffuse from the emulgel into the agar medium. Flavonoids may affect bacterial membrane integrity and cellular processes; alkaloids may interfere with essential cellular functions; saponins may alter membrane permeability; and tannins may bind proteins and inhibit certain enzymes. The combined effects of these compounds could contribute to bacterial growth inhibition (Febrianti *et al.*, 2022; Ulfa *et al.*, 2021). Emulgel serves as a topical carrier by incorporating the extract into a semisolid system that can be applied to the skin. Its emulsion component accommodates ingredients with different solubility characteristics, while its gel structure supports consistency and application. However, the

release and diffusion of active compounds depend on the formulation's composition and physical properties. The lower spreadability and reduced homogeneity of Formula 3 may therefore affect the uniformity of application and active-compound release, even though this formula produced the largest inhibition zones in the present assay. The observed inhibition should be attributed primarily to the extract-containing formulations rather than to a proven enhancement of antibacterial activity by the emulgel base itself.

Statistical analysis showed significant differences in antibacterial activity among treatment groups for both bacterial species ($p < 0.001$). For *S. epidermidis*, the data met the reported normality and variance-homogeneity assumptions, supporting the use of one-way ANOVA followed by Tukey's post hoc test. The reported comparison between Formulas 2 and 3 was significant ($p = 0.022$), indicating greater inhibition at the 20% concentration than at 15%. For *P. acnes*, the data were normally distributed but showed unequal variances (homogeneity test $p = 0.036$); therefore, Welch's ANOVA followed by the Games–Howell post hoc test would be more appropriate than standard one-way ANOVA with Tukey's test. Overall, Formula 3 (20%) showed the greatest antibacterial activity among the tested extract formulations, but its lower homogeneity and spreadability indicated that further optimization was needed. These results support the potential of Japanese papaya leaf extract emulgel as a topical antibacterial formulation, although further testing is required to establish formulation stability, skin compatibility, and efficacy under conditions relevant to clinical use.

Statistical analysis showed a significant difference in antibacterial activity for both bacteria ($p < 0.001$). *S. epidermidis* was analyzed using One-Way ANOVA, whereas *P. acnes* was analyzed using Welch ANOVA because the data were not homogeneous ($p = 0.036$).

CONCLUSION

Based on the results of this study, Japanese papaya (*Cnidoscolus aconitifolius*) leaf extract emulgel exhibited antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes*. Formula 3 (20% extract) demonstrated the highest antibacterial activity among the tested formulations. The negative control showed no inhibition zone, indicating that the antibacterial activity was associated with the presence of the leaf extract. However, statistical comparisons between each formulation and the negative control are required to confirm whether these differences were statistically significant ($p < 0.05$).

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