Pharmacy Reports

https://doi.org/10.51511/pr.112

ORIGINAL ARTICLE

Open Access

# Concentration-dependent antibacterial activity of *Ruellia tuberosa* L. leaf ethanol extract against *Propionibacterium acnes* ATCC 6919

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**Abstract:** Rising antibiotic resistance in *Propionibacterium acnes* necessitates alternative anti-acne agents. *Ruellia tuberosa* L. (Acanthaceae) possesses documented antibacterial potential, yet its activity against *P. acnes* remains unevaluated. This study investigated the concentration-dependent antibacterial activity of *R. tuberosa* leaf ethanol extract against *P. acnes* ATCC 6919. Leaves were extracted by maceration using 96% ethanol. Phytochemical classes were identified by thin-layer chromatography. Antibacterial activity was assessed using the Kirby-Bauer disc diffusion method in triplicate (n = 3) at concentrations of 15%, 20%, 25%, 30%, and 35% (w/v), with 0.1% clindamycin and distilled water as positive and negative controls, respectively. Data were analyzed by one-way ANOVA with Tukey's HSD post-hoc test. TLC confirmed alkaloids, flavonoids, tannins, saponins, and terpenoids. Inhibition zones increased from 5.00 ± 1.30 mm (15%, weak) to 11.82 ± 0.84 mm (35%, strong), compared to 22.05 ± 0.27 mm for clindamycin. Significant differences were detected among all groups ($p < 0.0001$). The 30% concentration was the lowest to achieve strong inhibitory activity, with no significant advantage conferred by increasing to 35%. *R. tuberosa* leaf ethanol extract exhibits concentration-dependent antibacterial activity against *P. acnes*, with 30% identified as the optimal concentration, warranting further investigation for botanical anti-acne formulation development.

**Keywords**: acne vulgaris, antibacterial activity, disc diffusion, *Propionibacterium acnes*, *Ruellia tuberosa*

## Introduction

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit and one of the most prevalent dermatological conditions worldwide, affecting an estimated 9.4% of the global population and ranking as the eighth most common disease globally [1,2]. Its burden falls disproportionately on adolescents and young adults: according to the Global Burden of Disease Study 2021, the age-standardized prevalence rate among individuals aged 10–24 years rose from 8,563 to 9,791 per 100,000 population between 1990 and 2021, representing an average annual increase of 0.43% [3]. Beyond physical morbidity, acne exerts substantial psychological consequences including diminished self-esteem, depression, and social withdrawal, contributing to a significant and growing disability-adjusted life year (DALY) burden [3,4]. In Lampung Province, Indonesia, local data indicate a higher prevalence in females (69.75%) than males (30.3%), with young adults aged 16–25 years accounting for 53.2% of cases [5], underscoring the regional public health relevance of this condition.

*Propionibacterium acnes* (recently reclassified as *Cutibacterium acnes*), a Gram-positive anaerobic bacterium, plays a central role in the pathogenesis of acne by colonizing pilosebaceous follicles and triggering inflammatory cascades through lipase-mediated hydrolysis of sebum triglycerides, production of pro-inflammatory mediators, and activation of innate immune responses via Toll-like receptor signaling [6,7]. Throughout this study, the name *P. acnes* is retained to maintain consistency with the ATCC 6919 strain designation used. Current treatment strategies encompass topical and systemic antibiotics (clindamycin, erythromycin, tetracyclines), topical retinoids, and benzoyl peroxide. However, prolonged antibiotic use has fueled a global rise in antibiotic-resistant *P. acnes* strains: a recent systematic review and meta-analysis reported that clindamycin resistance increased from 8% in 2008 to 42% by 2023, while erythromycin resistance reached 44% by 2024 [8,9]. Furthermore, many topical agents carry adverse effects including irritation, erythema, and photosensitivity, whereas systemic therapies such as isotretinoin are

associated with teratogenicity and hepatotoxicity risks [10]. These challenges collectively highlight the urgent need for alternative therapeutic agents from natural sources offering effective antibacterial activity with a reduced resistance burden.

*Ruellia tuberosa* L. (family Acanthaceae), commonly known as purple ruellia or minnieroot, is a perennial herbaceous plant native to tropical America that has naturalized throughout Southeast Asia, including Indonesia, where it grows abundantly as a wild plant [11]. The plant has an established ethnomedicinal history of use for treating fever, inflammation, wounds, and infections across tropical Asia [12]. A comprehensive review by Sharma et al. (2024) documented that *R. tuberosa* extracts and phytochemicals exhibit potent bioactivities including antimicrobial, anti-inflammatory, wound healing, and antidiabetic properties, attributed to a rich phytochemical profile encompassing alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds [11]. LC–MS/MS profiling has identified catechin, gallic acid, and ellagic acid as major constituents of *R. tuberosa* aerial extracts, compounds known to possess significant antibacterial properties [13]. These phytochemicals act through distinct yet complementary mechanisms—including membrane disruption, protein denaturation, inhibition of nucleic acid synthesis, and interference with cell wall assembly—suggesting that *R. tuberosa* extracts may exert antibacterial effects through synergistic multi-target mechanisms that could circumvent conventional resistance pathways.

Previous investigations have demonstrated the broad-spectrum antibacterial potential of *R. tuberosa*. Handayani et al. (2020) reported that ethanol extracts inhibited both Gram-positive and Gram-negative pathogenic bacteria [14], while Mundriyastutik et al. (2022) demonstrated efficacy against *Staphylococcus aureus*, a Gram-positive bacterium that shares structural features with *P. acnes*, particularly regarding thick peptidoglycan cell wall composition [15]. Phytochemical profiling studies using multiple extract fractions confirmed antibacterial activity against *Escherichia coli* with favorable in silico docking scores for key constituents against bacterial target proteins [16]. However, a critical research gap remains: systematic dose-response investigations of *R. tuberosa* leaf extract specifically targeting *P. acnes* are notably absent from the published literature, precluding evidence-based guidance on optimal concentrations for anti-acne formulation development.

The present study therefore systematically evaluates the concentration-dependent antibacterial activity of *R. tuberosa* leaf ethanol extract against *Propionibacterium acnes* ATCC 6919 using the standardized Kirby-Bauer disc diffusion method. Specific objectives are: (1) to determine antibacterial activity across a concentration range of 15%–35% (w/v); (2) to characterize the concentration-response relationship; (3) to identify the optimal extract concentration for maximal antibacterial efficacy; and (4) to characterize the major phytochemical constituents via thin-layer chromatography. This investigation aims to provide foundational in vitro evidence for *R. tuberosa* as a candidate botanical anti-acne agent, establishing a basis for future bioassay-guided fractionation, topical formulation development, and clinical efficacy trials.

## Methods

### Plant material and extract preparation

Fresh leaves of *Ruellia tuberosa* L. were collected from Bandar Lampung, Lampung Province, Indonesia. Leaves were washed thoroughly under running water, air-dried at room temperature away from direct sunlight for 14 days, and ground to a fine powder using an electric grinder. Powder moisture content was verified prior to extraction.

Maceration was selected as the extraction method due to its suitability for thermolabile phytochemicals, as prolonged heat exposure during extraction may degrade bioactive compounds such as flavonoids and tannins [17,18]. Extraction was performed using 96% ethanol, which as a polar solvent efficiently extracts a broad spectrum of polar and moderately polar secondary metabolites including flavonoids, tannins, alkaloids, saponins, and phenolic compounds [18,19]. Powdered leaf material (220 g) was mixed with 3,000 mL of 96% ethanol (1:13.6 w/v) and macerated at room temperature for 3 × 24 h with gentle agitation for 5 minutes every 24 h. After each maceration period, the mixture was vacuum-filtered through Whatman No. 1 filter paper, and the residue underwent two additional re-maceration cycles under identical conditions. Pooled filtrates were concentrated using a rotary vacuum evaporator at 50°C and 70 rpm to obtain the crude semi-solid extract, which was stored at 4°C in amber glass vials until use.

The absence of residual ethanol in the crude extract was verified prior to antibacterial testing using an ester formation test: 1 g of crude extract was mixed with

1 mL of concentrated $H_2SO_4$ and 1 mL of glacial $CH_3COOH$, sealed with a cotton plug, and heated at 90°C for 5 min. The absence of a characteristic fruity ester odor confirmed complete solvent evaporation, validating that subsequent antibacterial effects could be attributed solely to the phytochemical constituents of the extract.

### Phytochemical screening

Phytochemical screening was performed using thin-layer chromatography (TLC) on silica gel $F_{254}$ plates (1 × 7 cm). The TLC chamber was pre-saturated with the respective mobile phase for 30 min prior to each run. Extract samples were spotted at 1 cm from the bottom edge using a glass capillary, dried under ambient air, and eluted until the solvent front migrated approximately 6 cm. Plates were examined under UV light (254 nm and 366 nm), then sprayed with appropriate detection reagents and heated at 100–110°C for 3–5 min. Retention factor (Rf) values were calculated as the distance traveled by the compound divided by the distance traveled by the solvent front.

Phytochemical classes were detected using established protocols [20–23]. Alkaloids were detected using chloroform:methanol (9:1 v/v) as the mobile phase with Dragendorff reagent, producing characteristic orange-brown spots [20]. Flavonoids were resolved using *n*-butanol:acetic acid:water (4:1:5 v/v/v) with citroborate reagent, visualized as blue fluorescence under UV 366 nm [21]. Tannins were identified using chloroform:methanol (9:1 v/v) with 1% $FeCl_3$ reagent, yielding greenish-black coloration [22]. Saponins and terpenoids were detected using chloroform:methanol (9:1 v/v) with Liebermann-Burchard reagent, producing blue-green coloration [23].

### Test solutions and controls

A stock solution was prepared by dissolving 8.75 g of crude extract in 25 mL of sterile distilled water to yield a nominal concentration of 35% w/v. Serial dilutions were then prepared in sterile distilled water to obtain working concentrations of 15%, 20%, 25%, 30%, and 35% w/v. All solutions were prepared fresh on the day of use and stored at 4°C in sterile amber vials until application.

Sterile distilled water served as the negative control to confirm that the solvent vehicle contributed no intrinsic antibacterial activity. Clindamycin phosphate at 0.1% w/v was used as the positive control, given its established bacteriostatic and bactericidal activity against *P. acnes* via inhibition of the 50S ribosomal subunit [8]. The clindamycin solution was prepared by dissolving 0.1 g of clindamycin phosphate in 100 mL of sterile distilled water, stored in an amber glass bottle at 2–8°C, and used within 24 h of preparation.

### Bacterial strain and inoculum preparation

*Propionibacterium acnes* ATCC 6919 was maintained on nutrient agar slants at 4°C with monthly subculturing under anaerobic conditions. Prior to each experiment, the strain was subcultured onto fresh nutrient agar and incubated anaerobically at 37°C for 24–48 h to ensure viability.

Bacterial inocula were prepared by transferring 3–5 well-isolated colonies from a 24 h culture into 9 mL of sterile 0.9% NaCl using a sterile inoculating loop, followed by thorough vortexing for 10–15 s. Turbidity was adjusted to match the 0.5 McFarland standard (approximately $1.5 \times 10^8$ CFU/mL), verified spectrophotometrically at 625 nm (target absorbance: 0.08–0.10) [24]. The McFarland 0.5 standard was prepared by mixing 99.5 mL of 0.18 M $H_2SO_4$ with 0.5 mL of 0.048 M $BaCl_2$, with turbidity confirmed spectrophotometrically. The standard was stored in sealed dark glass vials at room temperature and renewed every two weeks.

### Media preparation

Nutrient agar slants for bacterial maintenance were prepared by dissolving 0.168 g of nutrient agar powder in 6 mL of distilled water, heating to complete dissolution, dispensing into sterile test tubes, and autoclaving at 121°C for 15 min at 15 psi. After cooling to approximately 50°C, tubes were positioned at a 30° angle for 30 min to solidify as slants and stored at 4°C.

Nutrient agar plates for antibacterial testing were prepared by dissolving 2.52 g of nutrient agar powder in 90 mL of distilled water, boiling to complete dissolution, and sterilizing by autoclaving at 121°C for 15 min at 15 psi. After equilibration to 45–50°C in a water bath, approximately 15 mL of medium was poured into each sterile 9 cm Petri dish and allowed to solidify for 30 min at room temperature. Plates not used immediately were stored inverted at 4°C and equilibrated to room temperature for 30 min before inoculation.

### Antibacterial activity assay

All inoculation procedures were performed under aseptic conditions in a laminar air flow cabinet previously decontaminated with 70% ethanol and UV-irradiated for 15 min. Plates were surface-inoculated with the standardized *P. acnes* suspension using a sterile cotton swab, which was streaked across the entire agar surface in three directions with the plate rotated 60° between each pass to ensure a confluent bacterial lawn [25]. Inoculated plates were allowed to rest for 5 min at room temperature before disk placement.

Sterile filter paper discs (Whatman No. 1, 6 mm diameter) were each impregnated with 20 µL of the respective test solution (15%, 20%, 25%, 30%, or 35% extract, positive control, or negative control) and allowed to absorb for 10 min. Discs were removed with sterile forceps, briefly blotted on sterile filter paper to remove excess liquid, and placed on the inoculated agar surface with a minimum center-to-center distance of 24 mm. A maximum of five discs were placed per 9 cm plate, with different concentrations distributed across separate plates to avoid diffusion interference. All plates were incubated in an anaerobic jar fitted with a gas-generating kit at 37°C for 24 h. Anaerobic conditions were verified using anaerobic indicator strips placed inside each jar.

Each treatment condition was tested in triplicate (n = 3) across three independent experimental runs performed on different days. After incubation, inhibition zones surrounding each disc were measured using a digital caliper with 0.01 mm precision. Four perpendicular diameter measurements (vertical, horizontal, and two diagonals) were taken per zone, and the mean diameter was recorded. Inhibitory activity was classified using the criteria of Davis and Stout [26]: no inhibition (0 mm), weak (1–5 mm), moderate (6–10 mm), strong (11–20 mm), and very strong (>20 mm).

### Statistical analysis

All data are expressed as mean ± standard deviation (SD) from three independent replicates. Statistical analyses were performed using GraphPad Prism 10 (Boston, USA). Prior to parametric testing, data normality was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene's test (both acceptable at $p > 0.05$). Where assumptions were met, one-way analysis of variance (ANOVA) was applied at a 95% confidence level ($\alpha = 0.05$) to detect significant differences among treatment groups. Post-hoc pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test. Differences were considered statistically significant at $p < 0.05$.

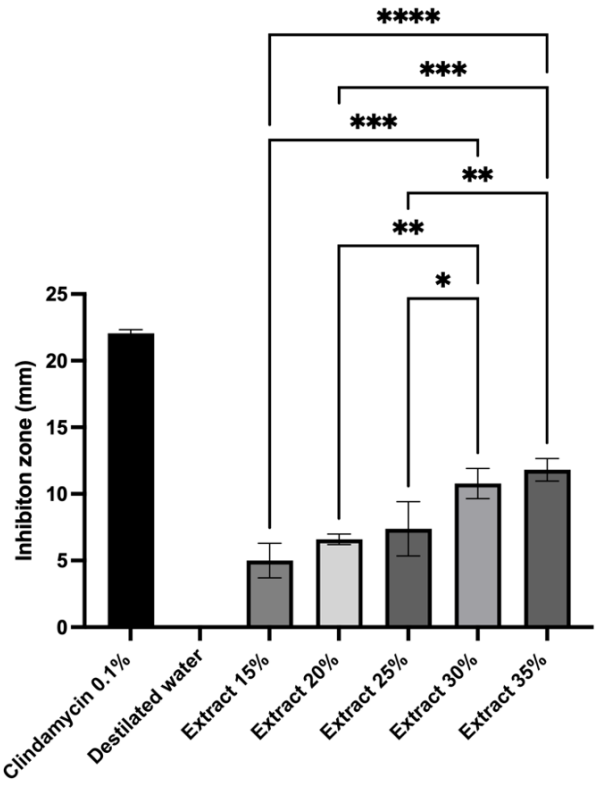


**Figure 1.** Mean inhibition zone diameters (mm) of *R. tuberosa* leaf ethanol extract and controls against *P. acnes* ATCC 6919 determined by the disc diffusion method. Data represent mean ± SD (n = 3). Different letters above bars indicate statistically significant differences between groups (Tukey's HSD, $p < 0.05$).

## Results

### Phytochemical characterization

Phytochemical screening by TLC confirmed the presence of five secondary metabolite classes in the *R. tuberosa* leaf ethanol extract (Table 1). Alkaloids were detected as orange-brown spots following Dragendorff reagent application; flavonoids exhibited blue fluorescence under UV 366 nm with citroborate reagent; tannins yielded greenish-black coloration with 1% $FeCl_3$; and both saponins and terpenoids produced blue-green coloration with Liebermann-Burchard reagent. The ethanol-free verification test produced no characteristic ester odor following acid treatment and heating, confirming complete solvent evaporation from the crude extract prior to antibacterial testing.

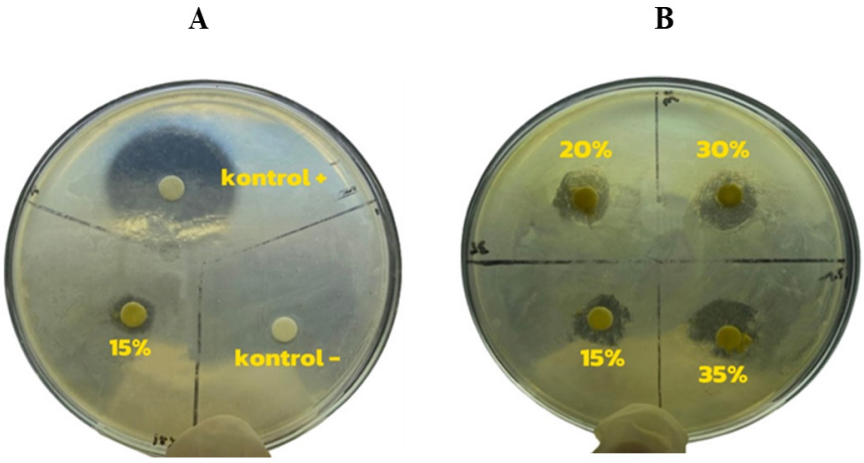


**Figure 2.** Representative disc diffusion plates showing antibacterial activity of *R. tuberosa* leaf ethanol extract against *P. acnes* ATCC 6919. (A) Positive control (clindamycin 0.1%), negative control (distilled water), and extract at 15% concentration. (B) Extract concentrations of 20%, 30%, and 35%. Clear zones of inhibition surrounding the discs indicate suppression of bacterial growth. The 25% concentration is not shown as panel B presents representative concentrations illustrating the dose-response trend across the tested range.

**Table 1.** Phytochemical screening results of *Ruellia tuberosa* L. leaf ethanol extract by TLC

| Compound class | Detection results | Visual observation |
|---|---|---|
| Alkaloid | Positive (+) | Orange-brown spots |
| Flavonoid | Positive (+) | Blue fluorescence (UV 366 nm) |
| Tannin | Positive (+) | Greenish-black coloration |
| Saponin | Positive (+) | Blue-green coloration |
| Terpenoid | Positive (+) | Blue-green coloration |

(+) = detected.

### Antibacterial activity against *Propionibacterium acnes*

The disc diffusion assay demonstrated concentration-dependent antibacterial activity of *R. tuberosa* leaf ethanol extract against *P. acnes* ATCC 6919 across all tested concentrations. Mean inhibition zone diameters for all treatment groups are presented in Table 2 and Figure 1, and representative plate images are shown in Figure 2.

The negative control (distilled water) produced no inhibition zone in any replicate, confirming that the solvent vehicle contributed no intrinsic antibacterial activity. The positive control (0.1% clindamycin) yielded a mean inhibition zone of 22.05 ± 0.27 mm, classified as very strong antibacterial activity according to the criteria of Davis and Stout [26], validating the susceptibility of the test organism and the reliability of the assay conditions.

Among the extract concentrations, inhibition zone diameters increased progressively with concentration, ranging from 5.00 ± 1.30 mm at 15% to 11.82 ± 0.84 mm at 35% (w/v). The 15% concentration produced weak inhibitory activity (1–5 mm), concentrations of 20% and 25% fell within the moderate category (6–10 mm), and concentrations of 30% and 35% both achieved strong inhibitory activity (11–20 mm) [26].

One-way ANOVA revealed statistically significant differences in inhibition zone diameters among all treatment groups ($F = 161.2$, $df = 6$, $p < 0.0001$). Post-hoc pairwise comparisons using Tukey's HSD test identified three statistically distinct groupings (Table 2). The positive control (clindamycin) differed significantly from all other groups ($p < 0.0001$). The negative control differed significantly from all extract concentrations ($p \leq 0.0008$). Among the extract concentrations, the 15%, 20%, and 25% concentrations were not significantly different from one another ($p = 0.5531$, 0.1635, and 0.9676, respectively), forming one homogeneous subgroup. The 30% and 35% concentrations were likewise not significantly different from each other ($p = 0.8908$), but both differed significantly from the 15%,

**Table 2.** Inhibition zone diameters (mm) of *R. tuberosa* leaf ethanol extract against *P. acnes* ATCC 6919 using the disc diffusion method (n = 3).

| Treatment Group | Mean ± SD (mm) | Inhibitory Category* |
|---|---|---|
| Negative control (distilled water) | 0 ± 0 | No inhibition |
| Positive control (clindamycin 0.1%) | 22.05 ± 0.27 | Very strong |
| Extract 15% | 5.00 ± 1.30 | Weak |
| Extract 20% | 6.60 ± 0.39 | Moderate |
| Extract 25% | 7.38 ± 2.03 | Moderate |
| Extract 30% | 10.78 ± 1.12 | Strong |
| Extract 35% | 11.81 ± 0.84 | Strong |

* Inhibitory strength categories per Davis and Stout [26]: none (0 mm), weak (1–5 mm), moderate (6–10 mm), strong (11–20 mm), very strong (>20 mm).

20%, and 25% concentrations ($p \leq 0.0216$). Notably, the 25% concentration differed significantly from both 30% ($p = 0.0216$) and 35% ($p = 0.0026$), marking the transition between the two extract subgroups. Complete pairwise comparison results are presented in Table 3.

## Discussion

This study demonstrated that ethanol extract of *R. tuberosa* leaves exerts concentration-dependent antibacterial activity against *P. acnes* ATCC 6919, with inhibition zones increasing progressively from 5.00 ± 1.30 mm at 15% to 11.82 ± 0.84 mm at 35% (w/v). The absence of inhibition in the negative control confirmed that the solvent vehicle contributed no intrinsic antibacterial activity, and the ethanol-free verification test confirmed that residual solvent was not responsible for the observed effects. The positive control (0.1% clindamycin) yielded 22.05 ± 0.27 mm, consistent with very strong activity against *P. acnes* [26], validating assay conditions and organism susceptibility. The lower value compared to the 38.33 mm reported by Putri et al. (2022) under nominally similar conditions may reflect differences in agar depth, anaerobic jar performance, inoculum preparation, or disc impregnation volume; however, both values fall within the very strong inhibitory category, confirming adequate assay performance.

The dose-response relationship observed across the full concentration range is consistent with established pharmacological principles governing concentration-dependent antimicrobial activity [27]. However, Tukey's HSD post-hoc analysis revealed a more nuanced pattern than a simple linear progression. Concentrations of 15%, 20%, and 25% were not significantly different from one another ($p = 0.1635–0.9676$), forming a statistically homogeneous subgroup, despite spanning both the weak and moderate inhibitory categories by Davis and Stout criteria [26]. In contrast, the 30% and 35% concentrations formed a second homogeneous subgroup ($p = 0.8908$), both significantly exceeding the lower concentration group ($p \leq 0.0216$). This pattern indicates a meaningful step-change in antibacterial efficacy between the 25% and 30% concentrations, rather than a gradual linear increase across all tested concentrations. From a practical standpoint, 30% represents the lowest concentration achieving strong inhibitory activity with no statistically significant advantage conferred by further increasing to 35%, suggesting it as the optimal concentration balancing efficacy and resource efficiency for potential formulation development.

The relatively high standard deviation at 25% (SD = 2.03 mm) warrants consideration. This variability likely reflects inherent biological heterogeneity in bacterial response at intermediate concentrations, where the extract transitions between weak-to-moderate and strong inhibitory regimes, as well as minor variation in agar diffusion at mid-range concentrations. The observation that 25% was statistically indistinguishable from both 15% and 20% further supports the interpretation that this concentration falls within a transitional zone of antibacterial activity.

TLC screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in the extract, consistent with previous phytochemical reports on *R. tuberosa* [11,14]. These compound classes are individually recognized to possess antibacterial properties through distinct mechanisms, including membrane disruption, protein denaturation, inhibition

of nucleic acid synthesis, and interference with cell wall assembly [28,29]. The thick peptidoglycan layer of Gram-positive bacteria such as *P. acnes* is a primary target for tannins and alkaloids in particular [29], which may partly account for the observed activity. However, since individual compounds were not isolated or quantified in this study, the relative contribution of each class and any potential synergistic interactions cannot be determined from the present data alone; these aspects require future bioassay-guided fractionation and mechanistic investigation.

The antibacterial activity observed here is consistent with prior reports on *R. tuberosa* against related Gram-positive bacteria. Mundriyastutik et al. (2022) demonstrated inhibitory activity of *R. tuberosa* ethanol extract against *Staphylococcus aureus* [15], a species sharing structural similarities with *P. acnes* in terms of cell wall composition. Handayani et al. (2020) similarly reported broad-spectrum activity against both Gram-positive and Gram-negative pathogens [14], while Sharma et al. (2023) confirmed antibacterial activity of *R. tuberosa* leaf fractions against *E. coli* with favorable in silico docking scores for key constituents [16]. The present findings extend this evidence base to *P. acnes* specifically, a clinically relevant target for which *R. tuberosa* had not previously been systematically evaluated across a concentration range.

While the highest extract concentration (35%) achieved strong inhibitory activity, the inhibition zone remained substantially lower than that of clindamycin. This difference is expected when comparing a crude multi-component extract with a purified pharmaceutical agent at an optimized dose, and does not preclude the potential utility of *R. tuberosa* as a botanical anti-acne agent, particularly given growing concerns over rising clindamycin resistance rates in *P. acnes* [8,9].

Several limitations of this study should be acknowledged. The in vitro disc diffusion method does not fully replicate the biological complexity of acne-affected skin, including sebum composition, pH gradients, and the stratum corneum barrier. Only a single reference strain (ATCC 6919) was tested; clinical *P. acnes* isolates, including antibiotic-resistant strains, may respond differently. The use of nutrient agar rather than more selective anaerobic media such as Brucella Blood Agar may have influenced growth characteristics and zone measurements. Phytochemical characterization was limited to class-level TLC screening without isolation, quantification, or direct mechanistic investigation. The concentration range tested (15–35% w/v) suggests that the minimum inhibitory concentration likely falls below 15%, as the lowest tested concentration already produced measurable inhibition; MIC determination would more precisely define the active threshold. No safety assessments, antibiofilm studies, or stability evaluations were conducted.

Future work should prioritize: (1) MIC and minimum bactericidal concentration (MBC) determination; (2) bioassay-guided fractionation to identify active constituents; (3) evaluation against clinical *P. acnes* isolates including antibiotic-resistant strains; (4) topical formulation development with safety assessment; and (5) in vivo validation in appropriate experimental models as a prerequisite for clinical translation.

## Conclusion

*Ruellia tuberosa* L. leaf ethanol extract exhibits concentration-dependent antibacterial activity against *Propionibacterium acnes* ATCC 6919. Inhibition zones ranged from 5.00 ± 1.30 mm at 15% (weak) to 11.81 ± 0.84 mm at 35% (strong), with statistically significant differences among all groups ($p < 0.0001$). The 30% concentration achieved strong inhibitory activity equivalent to that of 35% ($p = 0.8908$), identifying it as the optimal concentration balancing efficacy and economy. These findings support *R. tuberosa* as a candidate botanical anti-acne agent and provide a foundation for subsequent MIC determination, bioassay-guided fractionation, topical formulation development, and clinical evaluation.

## Acknowledgement

None.

## Funding

None

## Declaration of interest

The authors declare no competing interests.

Received: October 28, 2025
Revised: January 4, 2026
Accepted: January 5, 2026
Published: January 7, 2026

## Author contributions

Conceptualization, IY; Methodology and investigation, IY and LH; Data curation, LH and MLM; Formal analysis and writing—original draft, IY; Writing—review and editing, IY, LH, and MLM; Supervision, IY.

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