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RESEARCH ARTICLE

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Antibacterial activity of garlic ethanol extract (*Allium sativum Linn*) against *Propionibacterium acnes*

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ABSTRACT

Background: Acne vulgaris is a common dermatological condition largely associated with *Propionibacterium acnes* infection. The increasing resistance of *P. acnes* to conventional antibiotics necessitates alternative treatment approaches. Garlic (*Allium sativum* Linn) has documented antimicrobial properties, yet its specific activity against *P. acnes* remains underexplored.

Objective: To evaluate the antibacterial activity of garlic extract at various concentrations against *P. acnes* and determine its potential as an alternative acne treatment.

Methods: Garlic extract was prepared using ethanol maceration, followed by phytochemical screening. The antibacterial activity against P. acnes was assessed using the well diffusion method at concentrations of 10%, 40%, 70%, and 100%, with doxycycline and distilled water serving as positive and negative controls, respectively. Inhibition zones were measured and statistically analyzed.

Results: Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, saponins, and triterpenoids in the garlic extract. All tested concentrations exhibited significant antibacterial activity against *P. acnes*. The inhibition zone diameters were 32.83 mm (100%), 28.90 mm (70%), 26.60 mm (40%), and 15.29 mm (10%), compared to 38.81 mm for doxycycline, with statistically significant differences between all groups ($p<0.05$).

Conclusion: Garlic extract demonstrates potent antibacterial activity against *P. acnes*, with 70% concentration providing optimal efficacy relative to extract concentration, suggesting its potential as a natural alternative for acne treatment.

Keywords: Acne vulgaris, Allium sativum Linn, antibacterial activity, garlic extract, Propionibacterium acne

Introduction

Acne vulgaris represents one of the most common dermatological conditions worldwide, affecting approximately 9.4% of the global population [1] with regional prevalence rates reaching as high as 80-90% in countries like Indonesia [2]. This inflammatory condition of the pilosebaceous follicles, while not life-threatening, can significantly impact quality of life through psychological distress and permanent scarring [3]. Acne vulgaris ranks second among dermatologic conditions after dermatitis, contributing 1.79% to the global disease burden [4].

The pathogenesis of acne involves multiple factors, with bacterial infection playing a crucial role. *Propionibacterium acnes* (*P. acnes*), a gram-positive anaerobic bacillus, is a key microorganism implicated in acne development [5]. As part of the normal skin microbiota, P. acnes primarily colonizes the pilosebaceous unit, but under certain conditions, it can become pathogenic, triggering inflammation and subsequent acne formation [1]. Beyond acne, *P. acnes* has been associated with progressive macular hypomelanosis, medical device-related infections, sarcoidosis, and various

soft tissue infections, underscoring its clinical relevance [6].

The conventional treatment approach for acne typically involves antibiotics to control *P. acnes* proliferation [3]. However, the increasing prevalence of antibiotic resistance presents a significant challenge to effective treatment. Studies report alarming resistance rates of *P. acnes* to commonly prescribed antibiotics, with erythromycin resistance ranging from 45-91%, tetracycline resistance from 5-26.4%, and clindamycin resistance reaching 61.3% in Indonesia [2]. Additionally, prolonged antibiotic use can lead to adverse effects including skin discoloration, allergic reactions, and skin irritation [5]. These limitations highlight the urgent need for alternative antimicrobial agents with reduced potential for resistance development and minimal side effects.

Natural products have gained considerable attention as potential sources of novel antimicrobial compounds. Garlic (*Allium sativum* Linn) has been used for centuries in traditional medicine for its therapeutic properties [7]. Its rich phytochemical profile includes organosulfur compounds (approximately 2.3%), particularly allicin, which is formed when garlic is crushed or cut [8]. These compounds, along with other secondary metabolites such as tannins, flavonoids, alkaloids, saponins, and triterpenoids, contribute to garlic's documented antimicrobial effects against various pathogens [9,10].

Previous studies have demonstrated the efficacy of garlic extracts against gram-positive bacteria. Research on *Staphylococcus epidermidis*, another bacterium associated with acne, showed significant inhibition at concentrations ranging from 10-100%, with 70% concentration being most effective [11]. Similarly, studies on other Allium species have shown promising results against various bacteria [12,13]. However, despite the established antimicrobial properties of garlic and the clinical importance of P. acnes in acne pathogenesis, limited research has specifically examined the direct antibacterial activity of garlic extract against *P. acnes*.

This study aims to address this research gap by evaluating the antibacterial activity of various concentrations of garlic extract against *P. acnes*. By determining the minimum inhibitory concentration and assessing the potency of this natural agent, we seek to provide scientific evidence for garlic's potential as an alternative treatment for acne vulgaris, particularly in the context of increasing antibiotic resistance and the need for safer treatment options.

Method

Plant material collection and extraction

Garlic (*Allium sativum* Linn.) samples used in this study were obtained from Kuanoel Village, Fatumnasi District, South Central Timor Regency, East Nusa Tenggara Province. The extraction process began with four kilograms of fresh garlic bulbs that were separated from other onion structures. The garlic underwent wet sorting and was thoroughly washed under running water to remove dirt and contaminants. The clean garlic was then sliced thinly to increase surface area and dried in a closed room without direct sunlight for 7 days, with proper aeration to prevent moisture accumulation and fungal growth.

After drying, approximately 1.25 kg of dried garlic was obtained, which was then pulverized using a grinder to obtain 600 grams of simplisia powder. This powder was subjected to maceration by soaking it in 96% ethanol at a ratio of 1:5 (w/v) for 3 days, with daily stirring at the same hour to ensure consistent extraction. After the maceration period, the mixture was filtered to separate the liquid extract from the marc. The resulting liquid extract was then concentrated using a rotary vacuum evaporator at 40°C until a thick extract weighing 134 grams was obtained, representing an extraction yield of 22.3% [7,8].

Ethanol-free test and phytochemical screening

The thick garlic extract was tested for residual ethanol content by placing 1 mL of the extract into a test tube, then adding 2 drops of concentrated sulfuric acid (H_2SO_4) and 1 mL of potassium dichromate solution ($K_2Cr_2O_7$). The presence of

ethanol would be indicated by a color change from orange to bluish-green. In our extract, a mixed color was observed from the garlic extract solution and potassium dichromate solution with sulfuric acid, confirming the absence of ethanol [14].

Phytochemical screening was conducted to identify the presence of bioactive compounds in the garlic extract. The tests included:

- Tannins: 1 mL of extract was mixed with 2 mL of 5% $FeCl_3$ solution; a blue-black or green-black coloration indicated the presence of tannins.
- Flavonoids: 1 mL of extract was mixed with a few drops of 10% NaOH solution; a yellow coloration that disappeared upon addition of dilute HCl indicated flavonoids.
- Alkaloids: 1 mL of extract was mixed with 1 mL of Dragendorff's reagent; an orange-red precipitate indicated alkaloids.
- Saponins: 2 mL of extract was shaken vigorously with 5 mL of distilled water and allowed to stand; persistent froth indicated saponins.
- Triterpenoids: 1 mL of extract was dissolved in chloroform, and concentrated sulfuric acid was carefully added to form a layer; a reddish-brown coloration at the interface indicated triterpenoids [9,10].

Bacterial strain and culture conditions

Propionibacterium acnes bacterial samples were obtained from the Surabaya Health Laboratory Center, East Java, Indonesia. The confirmation of *P. acnes* was conducted using Gram staining [10]. The staining process involved several steps: (i) initial staining with crystal violet dye, (ii) mordant application with iodine to fix the dye, (iii) decolorization with ethanol to remove the dye from some bacteria, and (iv) counterstaining with safranin to stain decolorized bacteria pink. As expected for *P. acnes*, the bacteria retained the crystal violet dye and appeared purple under microscopic examination, confirming their Gram-positive nature. Furthermore, rod-shaped (bacillus) morphology was observed, which is characteristic of *P. acnes.*

The bacteria were maintained on Nutrient Agar (NA) plates and incubated anaerobically at 37°C for 24-48 hours prior to antibacterial testing.

Antibacterial activity assay

The antibacterial activity of garlic extract against P. acnes was evaluated using the agar well diffusion method [11,15]. A bacterial suspension was prepared by transferring 1-2 colonies of P. acnes using a sterile loop into 0.9% NaCl solution, adjusting the turbidity to match the 0.5 McFarland standard, approximately equivalent to 1.5×10^8 CFU/mL.

Using a sterile cotton swab, the bacterial suspension was evenly spread onto Nutrient Agar plates and allowed to stand for 30 minutes to ensure proper absorption of the inoculum. Wells of 6 mm diameter were then created in the center of each agar plate using a sterile cork borer. Four different concentrations of garlic extract (10%, 40%, 70%, and 100%) were prepared by diluting the stock extract with sterile distilled water. 50 µL of each extract concentration was placed into separate wells using a micropipette.

For comparison, doxycycline was used as a positive control and sterile distilled water as a negative control. All plates were incubated anaerobically at 37°C for 24 hours. After incubation, the diameter of the inhibition zone (clear area around each well) was measured in millimeters using a digital caliper. Four replicates were performed for each concentration and control to ensure reproducibility and statistical validity.

Statistical analysis

The antibacterial potency was categorized based on the diameter of the inhibition zone as described by Davis and Stout [16]: ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Data were analyzed using IBM SPSS Statistics software. Descriptive statistics were calculated to determine the mean and standard deviation of inhibition zone diameters for each concentration.

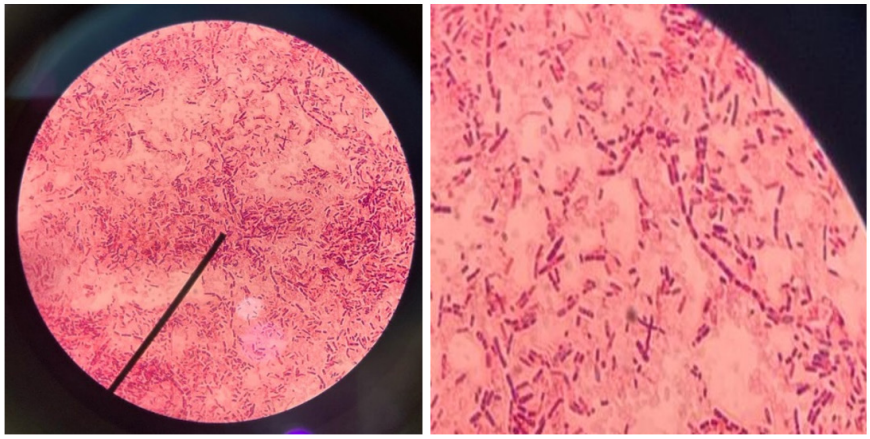

Figure 1. Gram staining results showing *P. acnes* under microscopic examination. Note the characteristic purple color and rod-shaped (bacillus) morphology, confirming gram-positive identification

To determine significant differences between treatment groups, One-Way Analysis of Variance (ANOVA) was conducted, with a significance level (α) of 0.05. Following a significant ANOVA result, post-hoc analysis using Tukey's Honestly Significant Difference (HSD) test was performed to identify which specific concentrations differed significantly from each other.

Results

Garlic extraction and phytochemical test

The extraction process yielded significant results, with 1.25 kg of dried garlic producing 600 grams of simplisia powder, which was subsequently processed to obtain 134 grams of thick extract. This represents an extraction yield of 22.3% from the simplisia powder, indicating an efficient extraction process. The ethanol-free test conducted on the thick extract showed a mixed color reaction when the extract was combined with potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4), confirming the absence of residual ethanol in the final extract [14].

Phytochemical screening revealed that the garlic extract contained a diverse profile of bioactive compounds with potential antibacterial activity. Specifically, the extract tested positive for tannins, flavonoids, alkaloids, saponins, and triterpenoids [9]. These secondary metabolites have been previously documented for their antimicrobial properties, with mechanisms ranging from cell membrane disruption to inhibition of bacterial protein synthesis [7].

Bacterial confirmation test

Gram staining was performed to confirm the identity of the test microorganism. Microscopic examination revealed purplish-blue stained bacteria with bacillus (rod) morphology (Figure 1), which is characteristic of gram-positive bacteria [5]. This observation, combined with the colony morphology and growth characteristics, confirmed that the bacteria used in this study were indeed *Propionibacterium acnes*, consistent with the expected features of this species [1].

Antibacterial test

The antibacterial activity of garlic extract against *P. acnes* was evaluated at four different concentrations: 10%, 40%, 70%, and 100%. All tested concentrations demonstrated inhibitory effects against *P. acnes*, as evidenced by the formation of clear inhibition zones around the wells (Figure 2). The diameter of these inhibition zones increased proportionally with higher extract concentrations, indicating a dose-dependent antibacterial effect.

The quantitative measurements of the inhibition zones are presented in Table 1. The highest inhibition was observed with the 100% extract

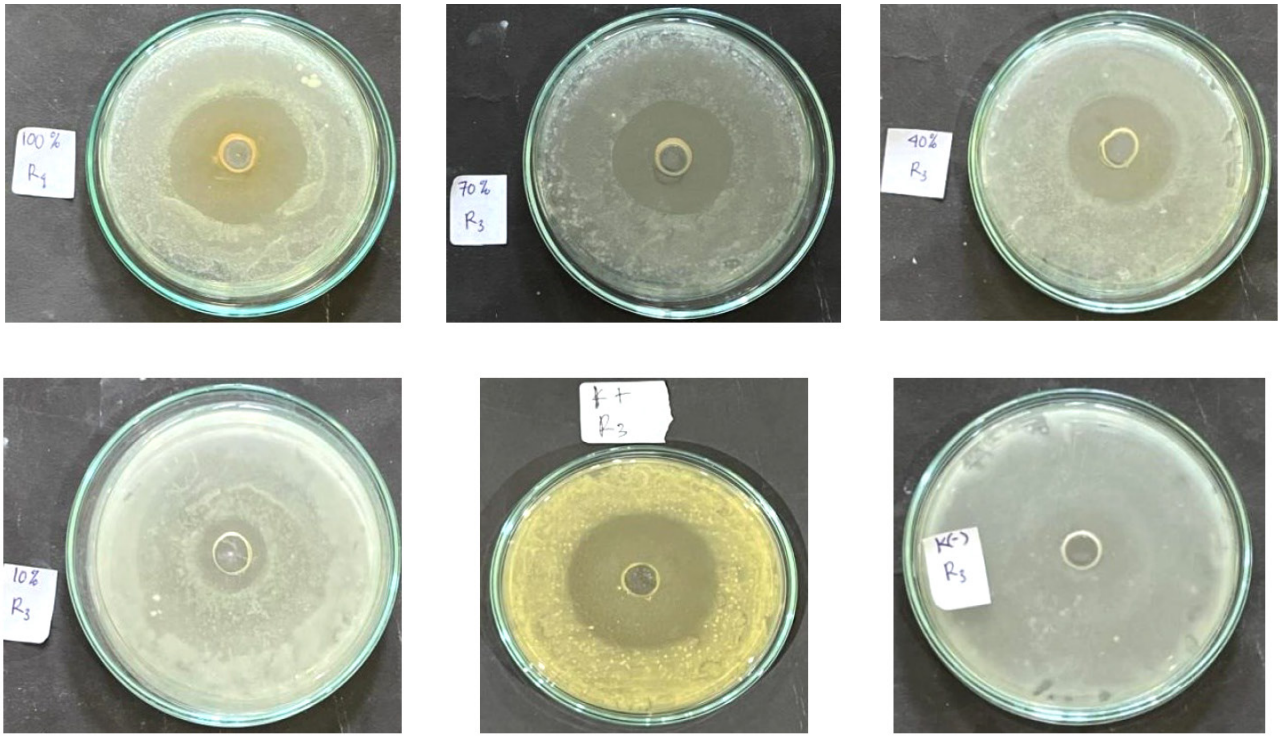

Figure 2. Antibacterial test results showing inhibition zones of garlic extract at different concentrations against *P. acnes*. Notice the clear zones surrounding the wells containing garlic extract at concentrations of A) 100%, B) 70%, C) 40%, D) 10%, E) Doxycycline (positive control), and F) Distilled water (negative control)

Table 1. The average diameter of the inhibition zone of garlic extract on the growth of *P. acnes*

Treatment	Average inhibition zone diameter (mm)	Potency
Doxycycline	38.81	Very strong
Extract 100%	32.83	Very strong
Extract 70%	28.90	Very strong
Extract 40%	26.60	Very strong
Extract 10%	15.29	Strong
Distilled water	0	None

concentration, producing an average inhibition zone diameter of 32.83 mm, which is classified as "very strong"" according to Davis and Stout criteria [16]. The 70% and 40% concentrations also exhibited very strong inhibitory effects with mean inhibition zone diameters of 28.90 mm and 26.60 mm, respectively. Even at the lowest tested concentration (10%), garlic extract maintained strong antibacterial activity with a mean inhibition zone of 15.29 mm. For comparison, the positive control (doxycycline) produced an inhibition zone of 38.81 mm, while the negative control (distilled water) showed no inhibitory effect.

Statistical analysis using One-Way ANOVA revealed a significant difference in the mean inhibition zone diameters between treatment groups ($p = 0.000$, $\alpha = 0.05$). Further post hoc analysis with the Tukey test demonstrated that each treatment group had a statistically significant difference in mean inhibition zone diameter compared to all other groups ($p < 0.05$). These findings confirm that the observed differences in antibacterial activity across the different extract concentrations were not due to chance but represent genuine variations in efficacy.

Discussion

This study examined the antibacterial activity of garlic (*Allium sativum* Linn) extract against *Propionibacterium acnes*, a key bacterial species implicated in acne vulgaris pathogenesis. Our findings demonstrate that garlic extract possesses significant antibacterial activity against *P. acnes*, with efficacy varying according to concentration.

The antibacterial assessment revealed a clear dose-dependent effect of garlic extract against *P. acnes*. All tested concentrations (10%, 40%, 70%, and 100%) exhibited inhibitory effects, with inhibition zone diameters ranging from 15.29 mm to 32.83 mm. According to Davis and Stout criteria [16], concentrations of 40%, 70%, and 100% demonstrated "very strong" antibacterial activity (inhibition zones ≥20 mm), while even the lowest concentration (10%) maintained "strong" activity (inhibition zone of 15.29 mm).

When compared with doxycycline, a first-line antibiotic for acne treatment, the 100% garlic extract produced an inhibition zone (32.83 mm) that was approximately 85% as effective as doxycycline (38.81 mm). This finding is particularly noteworthy, as doxycycline is considered highly effective against *P. acnes*, with reported sensitivity rates of 100% in previous studies [2]. The substantial antibacterial potency of garlic extract suggests it could potentially serve as a natural alternative in acne management strategies.

It is worth noting that the 70% concentration demonstrated high efficacy (28.90 mm inhibition zone) despite using a lower concentration than the 100% extract. This makes the 70% concentration potentially more practical from a resource utilization perspective, as it achieves very strong antibacterial effects with less raw material. This finding aligns with previous research by Indrayati and Diana (2020), who similarly found 70% garlic solution to be most effective against *Staphylococcus epidermidis*, another gram-positive bacterium associated with acne [11].

The phytochemical screening revealed that garlic extract contains tannins, flavonoids, alkaloids, saponins, and triterpenoids, consistent with findings from previous studies [8–10]. These bioactive compounds likely contribute to the observed antibacterial effects through various mechanisms. Allicin, the principal bioactive compound in garlic, is formed when garlic cells are damaged and the enzyme alliinase converts alliin to allicin [7]. Allicin exhibits antibacterial properties by inhibiting sulfhydryl-containing enzymes in bacteria, disrupting bacterial metabolism, and damaging cell membranes [17]. This mechanism is particularly effective against gram-positive bacteria like *P. acnes*.

Additionally, the identified flavonoids and tannins may enhance the antibacterial activity through different pathways. Flavonoids can form complexes with bacterial cell walls and disrupt membrane integrity, while tannins may precipitate bacterial proteins and inhibit bacterial enzymes [18]. Saponins likely contribute by forming pores in bacterial membranes, leading to cell lysis [19].

The synergistic action of these compounds may explain the robust antibacterial effect observed in our study. This is supported by El-Sayed et al. (2017), who found that garlic essential oils contain various organosulfur compounds with significant antimicrobial activity [7].

Our findings expand upon existing literature regarding garlic's antimicrobial properties. While numerous studies have demonstrated garlic's efficacy against various bacteria, specific research on its activity against *P. acnes* has been limited until now. The results of our study showed higher inhibition zones compared to research conducted by Indrayati et al. (2020), who tested garlic solution against *S. epidermidis*. In their study, even the 100% concentration did not reach the "very strong" category (≥20 mm inhibition zone), whereas in our study, even the 40% concentration achieved this level of efficacy against *P. acnes* [11].

The strong antibacterial activity of garlic extract against *P. acnes* suggests significant potential for its application in acne treatment. Natural products like garlic offer several advantages over conventional antibiotics, including reduced risk of bacterial resistance development and fewer side

effects. The emergence of antibiotic-resistant *P. acnes* strains poses a significant challenge in acne management. Studies have reported resistance rates of *P. acnes* to erythromycin (45-91%), tetracycline (5-26.4%), and clindamycin (61.3% in Indonesia) [5]. Therefore, exploring natural alternatives with multiple bioactive compounds and different mechanisms of action, such as garlic extract, could be valuable in addressing antibiotic resistance issues.

Furthermore, garlic extract could be incorporated into various formulations, including topical gels, creams, or masks, for direct application to acne-affected areas. Similar approaches have shown promise, as demonstrated by Sheikh et al. (2024), who developed an antimicrobial herbal cream containing garlic extract with significant efficacy [20].

Despite the promising results, this study has several limitations. First, as an in vitro investigation, it does not account for factors such as skin penetration, stability, and potential irritation that might affect clinical efficacy. Second, the antibacterial activity was tested against a single strain of *P. acnes*, whereas multiple strains with varying characteristics exist in clinical settings. Future research should focus on evaluating the stability and skin penetration of garlic extract in various formulations and onducting controlled clinical trials to assess the efficacy and tolerability of garlic extract-based preparations in acne patients.

Conclusion

This study demonstrated that garlic (*Allium sativum* Linn) extract possesses potent antibacterial activity against *Propionibacterium acnes*, a primary bacterium implicated in acne vulgaris pathogenesis. All tested concentrations (10%, 40%, 70%, and 100%) inhibited *P. acnes* growth, with concentrations of 40% and above showing "very strong" antibacterial activity.

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Author contributions

SDDWMS: Conceptualization, Investigation, Methodology, Writing – Original Draft. RLN: Supervision, Validation, Resources, Writing – Review & Editing. RMH: Formal Analysis, Methodology, Visualization, Writing – Review & Editing. TDN: Supervision, Project Administration, Funding Acquisition, Writing – Review & Editing.

Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public, or academic party related to the information contained in this manuscript.

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