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The Effect of Solvent Type and Extract Concentration of Binahong Leaves (*Anredera cordifolia* (Ten.) Steenis) on the In Vitro Antibacterial Activity against *Cutibacterium acnes*

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ABSTRACT

Acne is an inflammatory disorder of the pilosebaceous unit in which Cutibacterium acnes may contribute to disease-associated inflammation. Increasing antimicrobial resistance has encouraged the exploration of medicinal plants as potential sources of antibacterial agents, including binahong leaves (Anredera cordifolia (Ten.) Steenis). This study compared the in vitro antibacterial activity of 96% ethanol and ethyl acetate extracts of binahong leaves against C. acnes. A laboratory experimental study was conducted using 96% ethanol and ethyl acetate extracts at concentrations of 2%, 4%, and 6% w/v, with three replicates for each treatment. Antibacterial activity was assessed using a disk diffusion assay, with 1% Na-CMC and clindamycin 30 ppm as negative and positive controls, respectively. Inhibition zones were measured after incubation at 37 °C for 24 h. Data from the six extract groups were analyzed using two-way ANOVA followed by Tukey's post hoc test. Both solvent type ($p = 0.0002$) and extract concentration ($p < 0.001$) significantly affected inhibition-zone diameter, whereas their interaction was not significant ($p = 0.0878$). Ethyl acetate extract produced larger inhibition zones than 96% ethanol extract at 4% and 6% concentrations ($p = 0.017$ for each comparison), whereas no significant difference was observed at 2% ($p = 0.909$). The largest inhibition zone among the extracts was observed with 6% ethyl acetate extract (18.33 ± 1.53 mm), although this remained lower than that of clindamycin (31.67 ± 0.58 mm). These findings indicate that ethyl acetate extract of binahong leaves has greater in vitro inhibitory activity against C. acnes than the 96% ethanol extract at higher concentrations tested. However, the findings should be considered preliminary and require confirmation using standardized anaerobic conditions, MIC/MBC determination, and chemical standardization of the extract.

1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit characterized by the formation of comedones, papules, pustules, nodules, and, in some cases, scarring. Its pathogenesis involves complex interactions among follicular hyperkeratinization, increased sebum production, inflammatory responses, and alterations in the skin microbiota. In this process, *Cutibacterium acnes* plays an important role, not simply as a bacterium that colonizes the skin, but as a member of the skin microbiota that, under certain conditions, may contribute to inflammation within acne lesions. Therefore, controlling *C. acnes* remains an important consideration in the development of acne therapies, although modern treatment approaches are not intended to eradicate the entire population of this bacterium (Reynolds et al., 2024).

Cutibacterium acnes, formerly known as *Propionibacterium acnes*, is a Gram-positive bacterium that is normally present on human skin. Its role in acne is strain-dependent, as not all strains exhibit the same biological characteristics. Genomic studies have demonstrated differences in genetic characteristics between isolates associated with skin colonization and those associated with infection or inflammation (Podbielski et al., 2024). These differences indicate that the contribution of *C. acnes* to acne cannot be understood solely based on its presence on the skin, but should also be considered in relation to strain characteristics and interactions with the skin

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environment. Therefore, evaluating the antibacterial activity against *C. acnes* remains relevant as an initial step in assessing candidate agents with the potential to inhibit its growth.

The use of antibiotics in acne management faces challenges related to antimicrobial resistance and the ability of some *C. acnes* isolates to form biofilms. Resistance to several antibiotics commonly used in acne treatment, including macrolides and clindamycin, has been reported in clinical isolates (Paul et al., 2025; Bhadade et al., 2026). These findings highlight the need to explore new antibacterial candidates. However, antibiotic resistance does not, by itself, justify the use of natural products as substitutes for standard therapy (Hasan *et al.,* 2025). Rather, natural products should be considered a potential source of antibacterial candidates that require systematic evaluation through activity testing, material standardization, compound characterization, and further assessment of safety and efficacy.

One natural product with potential for development as an antibacterial candidate is binahong leaf (*Anredera cordifolia* (Ten.) Steenis). The selection of binahong leaves is supported by reports of their secondary metabolites, including flavonoids, tannins, saponins, triterpenoids, and phenolic compounds (Hasan *et al.,* 2025). Previous studies have demonstrated measurable flavonoid content in the ethyl acetate fraction of binahong leaves, while analysis of the ethanol extract has revealed several classes of secondary metabolites (Budiana, 2022; Rusdiana et al., 2024; Sugiaman et al., 2024). These metabolites may theoretically contribute to antibacterial activity through their effects on the structure and function of bacterial cellular components. However, the relationship between specific metabolite classes and activity against *C. acnes* cannot be established solely on the basis of phytochemical screening; therefore, their antibacterial activity needs to be confirmed through experimental testing.

The choice of solvent is one of the factors that can influence the chemical composition and biological activity of an extract (Wahyudin et al., 2025; Massi et al., 2017). Ethanol 96% has the ability to extract compounds across a relatively broad range of polarities, whereas ethyl acetate tends to extract semipolar constituents. These differences may result in distinct metabolite profiles and, consequently, influence the antibacterial activity of the resulting extracts. In binahong leaves, measurable flavonoid content has been reported in the ethyl acetate fraction, whereas ethanol extracts have been shown to contain several classes of secondary metabolites (Budiana, 2022; Rusdiana et al., 2024; Sugiaman et al., 2024). These findings provide a rationale for comparing extracts obtained using solvents with different polarity characteristics. However, evidence regarding differences in the activity of these two types of extracts against *C. acnes* at equivalent concentrations remains limited.

Previous studies have reported antibacterial activity of ethanol extracts of binahong leaves against *C. acnes* or *Propionibacterium acnes* (Waris & Indarto, 2023; Prantika et al., 2024). These findings provide preliminary evidence of the potential of binahong leaf ethanol extracts against bacteria associated with acne. However, studies directly comparing 96% ethanol and ethyl acetate extracts of binahong leaves against *C. acnes* at equivalent concentrations remain limited. Thus, an important knowledge gap remains regarding whether differences in solvent type, which produce extracts with distinct chemical characteristics, are associated with differences in the inhibitory activity against *C. acnes* at equivalent concentrations.

Based on this research gap, the novelty of the present study lies in the direct comparison of the antibacterial activity of 96% ethanol and ethyl acetate extracts of binahong leaves against *C. acnes* at equivalent concentrations of 2%, 4%, and 6% w/v. This approach allows the effects of solvent type and extract concentration on the diameter of the inhibition zone to be evaluated under the same experimental conditions. A negative control consisting of 1% Na-CMC and a positive control consisting of clindamycin at 30 ppm were included to facilitate the interpretation of the inhibitory activity produced by the two extracts.

This study aimed to evaluate the effects of extract type, namely 96% ethanol and ethyl acetate extracts of binahong leaves, and extract concentrations of 2%, 4%, and 6% w/v on the inhibition zone diameter of *C. acnes* in vitro, as well as to compare the antibacterial activity of the two extracts at equivalent concentrations. The hypothesis was that solvent type and extract concentration would affect the inhibition zone diameter of *C. acnes*. The findings are expected to provide preliminary evidence regarding which extract shows greater potential for further

investigation, including determination of the minimum inhibitory concentration and minimum bactericidal concentration, standardization of the chemical profile and marker compounds, safety evaluation, and further assessment using standardized microbiological methods.

2. METHOD

This laboratory-based experimental study employed a factorial design consisting of two solvent types (96% ethanol and ethyl acetate) and three extract concentrations (2%, 4%, and 6% w/v). The study was conducted in February 2026 at the Microbiology Laboratory, Politeknik Sandi Karsa. Each treatment group was tested in triplicate. Binahong leaves were collected from Sungguminasa, Somba Opu District, South Sulawesi, Indonesia. The leaves were sorted to remove damaged parts and impurities, washed under running water, cut into smaller pieces, and dried at room temperature away from direct sunlight until a dry crude drug (simplisia) was obtained. The dried material was then pulverized into powder prior to extraction.

For each extraction, 100 g of powdered binahong leaves was placed in a maceration vessel and soaked in either 96% ethanol or ethyl acetate at a material-to-solvent ratio of 1:7.5 (w/v). Maceration was performed for five days in a closed container protected from light, with periodic stirring. The filtrate was separated from the residue, and the residue was re-macerated for a total of three extraction cycles. The combined filtrates were concentrated using a rotary evaporator and further evaporated until a thick extract was obtained. The extracts were stored in closed containers until further testing. The extracts were prepared at concentrations of 2%, 4%, and 6% w/v by weighing 0.2, 0.4, and 0.6 g of extract, respectively, and dispersing each in 1% Na-CMC to a final volume of 10 mL. A 1% Na-CMC solution was used as the negative control. The positive control was prepared using clindamycin at a final concentration of 30 ppm, according to the original study protocol.

A pure culture of Cutibacterium acnes was used as the test microorganism. The bacterial culture was subcultured on Nutrient Agar, and the bacterial suspension was prepared in physiological saline and adjusted to a 0.5 McFarland standard. Antibacterial activity was evaluated using the disk diffusion method. Sterile Nutrient Agar was aseptically poured into Petri dishes and allowed to solidify. The bacterial suspension was evenly inoculated onto the surface of the agar. Paper disks impregnated with the respective extract solutions, negative control, or positive control were aseptically placed on the inoculated agar surface. The plates were incubated at 37 °C for 24 h according to the original study protocol. The diameter of the clear inhibition zones surrounding the disks was measured in millimeters.

Data were expressed as mean ± standard deviation (SD). The six extract treatment groups were reanalyzed from the raw data using two-way analysis of variance (ANOVA) to assess the effects of solvent type, extract concentration, and their interaction on the inhibition zone diameter. The normality of the residuals was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. When a statistically significant effect was identified, pairwise comparisons were performed using Tukey's post hoc test. Statistical significance was set at $p < 0.05$. The positive and negative controls were presented descriptively because they were not included in the primary factorial design.

3. RESULT AND DISCUSSION

Result

Table 1. Inhibition Zone Diameters of Binahong Leaf Extracts and Control Groups

Groups	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean ± SD (mm)
Negatif Control (Na-CMC 1%)	6	6	7	6,33 ± 0,58
Etil asetat 2%	13	12	12	12,33 ± 0,58
Etil asetat 4%	16	17	16	16,33 ± 0,58

Groups	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean ± SD (mm)
Etil asetat 6%	18	20	17	18,33 ± 1,53
Etanol 96% 2%	12	11	12	11,67 ± 0,58
Etanol 96% 4%	14	14	13	13,67 ± 0,58
Etanol 96% 6%	16	15	16	15,67 ± 0,58
Positif Control (clindamisin 30 ppm)	32	31	32	31,67 ± 0,58

Source: Primary research data (2026)

The inhibition zone diameter increased with increasing extract concentration for both extracts. At each concentration level, the mean inhibition zone produced by the ethyl acetate extract was greater than that of the ethanol extract. Among the extract-treated groups, the largest inhibition zone was observed with the 6% ethyl acetate extract, measuring 18.33 ± 1.53 mm, whereas the positive control produced an inhibition zone of 31.67 ± 0.58 mm.

Table 2. Results of Two-Way ANOVA for the Extract Treatment Groups

Source of Variation	df	F	p
Solvent type	1	27,00	0,0002
Concentration	2	57,00	<0,001
Solvent type × concentration	2	3,00	0,0878
Galat	12	-	-

The residuals met the assumption of normality (Shapiro–Wilk, p = 0.195), and homogeneity of variance across the extract groups was satisfied (Levene's test, p = 0.771).

Two-way ANOVA showed that both solvent type and extract concentration had significant effects on the inhibition zone diameter, whereas their interaction was not statistically significant. Tukey's post hoc test showed no significant difference between the ethyl acetate and ethanol extracts at a concentration of 2% (p = 0.909). At concentrations of 4% and 6%, the ethyl acetate extract produced significantly larger inhibition zones than the ethanol extract, with p = 0.017 for both concentrations.

Discussion

The findings showed that increasing the extract concentration was associated with an increase in the inhibition zone diameter for both solvents. For the ethyl acetate extract, the inhibition zone diameter increased from 12.33 ± 0.58 mm at 2% to 16.33 ± 0.58 mm at 4% and 18.33 ± 1.53 mm at 6%. A similar pattern was observed for the 96% ethanol extract, with inhibition zone diameters of 11.67 ± 0.58, 13.67 ± 0.58, and 15.67 ± 0.58 mm, respectively. This pattern indicates a concentration-dependent response within the range tested. Theoretically, increasing the extract concentration may increase the amount of compounds available to diffuse from the disk into the agar medium. However, inhibition zone diameter in a disk diffusion assay is determined not only by antibacterial activity but also by factors such as compound solubility, molecular size, extract viscosity, diffusion capacity in agar, and the amount of extract applied to the disk. Therefore, the increase in inhibition zone diameter should be interpreted as greater growth-inhibitory activity under the experimental conditions rather than as definitive evidence of increased antibacterial potency.

The difference in activity between the two extracts became more apparent at concentrations of 4% and 6%. The ethyl acetate extract produced inhibition zones of 16.33 ± 0.58

mm at 4% and 18.33 ± 1.53 mm at 6%, whereas the corresponding values for the 96% ethanol extract were 13.67 ± 0.58 and 15.67 ± 0.58 mm, respectively. Tukey's post hoc test showed no significant difference between the two extracts at 2% ($p = 0.909$), whereas significant differences were observed at both 4% and 6% ($p = 0.017$ for each concentration). These findings may indicate that the constituents extracted by ethyl acetate contributed more substantially to the inhibition of C. acnes growth at the higher concentrations tested. Ethyl acetate, a semipolar solvent, tends to extract compounds within a specific polarity range, whereas 96% ethanol has a broader extraction capacity. Budiana (2022) reported measurable total flavonoid content in the ethyl acetate fraction of binahong leaves, while ethanol extracts of binahong leaves have been reported to contain several classes of secondary metabolites, including flavonoids, tannins, saponins, and triterpenoids (Sugiaman et al., 2024). Differences in extract composition may therefore contribute to the observed differences in antibacterial activity. However, because this study did not perform chromatographic profiling or quantitative determination of marker compounds, the contribution of specific compounds to the observed antibacterial activity cannot be established.

The absence of a significant difference between the ethyl acetate and 96% ethanol extracts at a concentration of 2% is also noteworthy. At a relatively low concentration, the amount of active constituents available on the disks may not have been sufficient to produce a difference in response that could be detected statistically. In contrast, at concentrations of 4% and 6%, the difference in activity became more apparent, suggesting that the contribution of constituents present in the respective extracts may be more readily reflected at higher concentrations. This interpretation should nevertheless be made cautiously because the concentrations of active compounds in each extract were not determined. Therefore, the observed differences are more appropriately attributed to the overall characteristics of the extracts rather than to any single compound.

Two-way ANOVA showed that solvent type had a significant effect on the inhibition zone diameter ($p = 0.0002$), as did extract concentration ($p < 0.001$). In contrast, the interaction between solvent type and concentration was not statistically significant ($p = 0.0878$). These findings indicate that, within the tested concentration range of 2–6%, there was insufficient statistical evidence that the effect of concentration differed according to solvent type. In other words, increasing the extract concentration tended to increase the inhibition zone diameter for both extracts, while the ethyl acetate extract generally produced larger inhibition zones. Therefore, the findings are more appropriately interpreted in terms of the main effects of solvent type and concentration, without concluding that there was an interaction between the two factors.

The antibacterial activity of the 96% ethanol extract observed in this study was generally consistent with the findings of Waris and Indarto (2023), who reported inhibition zones produced by binahong leaf ethanol extract against Propionibacterium acnes. The present findings can also be compared with those of Prantika et al. (2024), who reported the activity of binahong leaf ethanol extract against Staphylococcus epidermidis and Propionibacterium acnes and determined the minimum inhibitory concentration using a dilution method. However, direct comparison of inhibition zone diameters across studies should be made with caution because of differences in extract type and concentration, test methods, bacterial strains, culture media, and incubation conditions. These factors can affect bacterial growth as well as the ability of extract constituents to diffuse through the medium. Therefore, differences in inhibition zone diameters do not necessarily reflect differences in intrinsic antibacterial potency.

The findings for the negative control should also be considered when interpreting the results. The 1% Na-CMC control yielded a measured value of 6.33 ± 0.58 mm. This value is very close to the diameter of the paper disk used [adjust according to the actual disk diameter]. Therefore, it should be clarified whether this measurement represents the disk diameter itself or indicates the formation of an inhibition zone extending beyond the disk. If the value corresponds only to the disk diameter, 1% Na-CMC did not exhibit meaningful inhibitory activity. Conversely, if a clear zone was observed beyond the disk diameter, this should be explicitly described because it may affect the interpretation of the extract activity. This clarification is important because the negative control is intended to confirm that the observed inhibitory effect is attributable to the extract rather than to its vehicle.

The positive control, clindamycin at 30 ppm, produced an inhibition zone diameter of 31.67 ± 0.58 mm, which was greater than that observed for all extract groups. This finding indicates that, under the experimental conditions used, the inhibitory activity of the extracts did not reach that of the positive control. However, this does not preclude the potential of the extracts for further investigation; rather, it indicates that the observed activity was lower than the response produced by clindamycin under the present test conditions. At the same time, the use of antibiotics for acne management should take antimicrobial resistance into consideration. Paul et al. (2025) reported resistance to several antibiotics among C. acnes isolates, while Bhadade et al. (2026) reported multidrug resistance in some isolates. These findings support the importance of rational antibiotic use and the exploration of new antibacterial candidates. However, they should not be interpreted as evidence that binahong leaf extracts can replace antibiotics with established clinical efficacy.

Biologically, the antibacterial activity of binahong leaf extracts may be associated with the presence of various secondary metabolites (Nuswantoro *et al.*, 2025). Flavonoids and phenolic compounds can generally interact with bacterial membrane components and cellular proteins, whereas tannins may interact with proteins and enzymes. Saponins may also theoretically affect cell membrane integrity, while triterpenoids may interact with lipid components of the membrane. These mechanisms provide a theoretical basis for the potential antibacterial activity of binahong leaf extracts. However, these mechanisms could not be directly demonstrated in the present study because compound isolation, identification of the active constituents, and molecular mechanism assays were not performed. Therefore, the findings are more appropriately attributed to the overall activity of the extract rather than to the action of any specific compound.

The interpretation of the findings is also limited by the use of the disk diffusion method. For plant extracts, the diameter of the inhibition zone reflects the combined effects of the antibacterial activity of the compounds and their ability to diffuse through the medium. Compounds with strong antibacterial activity but limited diffusion capacity may produce relatively small inhibition zones. Conversely, compounds that diffuse more readily may produce wider inhibition zones without necessarily exhibiting greater antibacterial activity. Therefore, differences in inhibition zone diameters between the ethyl acetate and 96% ethanol extracts should not be regarded as an absolute measure of antibacterial potency. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a dilution method is needed to provide a more specific measure of antibacterial activity.

The conditions used for testing C. acnes also represent an important limitation of this study. C. acnes has growth requirements related to oxygen availability and requires appropriate culture conditions to achieve optimal growth. In the documented procedure, Nutrient Agar and incubation at 37 °C for 24 h were used; however, information regarding the bacterial strain and incubation atmosphere was not fully documented. These factors should be considered because variations in strain, culture medium, atmosphere, and incubation duration may affect the growth of C. acnes and its response to the test substances. In addition, CLSI M11 recommends dilution methods as a reference approach for susceptibility testing of anaerobic bacteria and does not standardize disk diffusion as the primary method for such organisms. Therefore, the findings of this study should be regarded as preliminary screening data and should not be used to establish therapeutic concentrations or clinical efficacy.

An increase in inhibition zone diameter up to a concentration of 6% indicates that the highest concentration tested produced the greatest inhibitory response for both extracts. However, this finding does not establish that 6% is the optimal or effective concentration. Only three concentration levels were evaluated in this study; therefore, the response pattern at lower or higher concentrations remains unknown. In addition, inhibition zone diameter does not directly indicate the antibacterial concentration required to inhibit or kill the bacteria. Further studies using a broader concentration range, together with determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), are needed to provide a more comprehensive understanding of the concentration–activity relationship.

Overall, this study demonstrated that both ethyl acetate and 96% ethanol extracts of binahong leaves exhibited inhibitory activity against C. acnes under the test conditions used, with the ethyl acetate extract showing greater activity, particularly at concentrations of 4% and 6%. These findings provide preliminary evidence that solvent type may be an important factor to consider in the development of binahong leaf extracts as antibacterial candidates. However, in vitro activity against C. acnes cannot be equated with therapeutic efficacy against acne vulgaris, as acne pathogenesis also involves sebum production, follicular hyperkeratinization, inflammatory responses, and complex interactions within the skin microbiota (Inggas *et al*., 2025). Therefore, the present findings do not provide sufficient evidence to establish therapeutic efficacy as an anti-acne preparation.

Further studies should employ well-identified and documented *C. acnes* strains, appropriate and validated culture conditions, and more standardized antimicrobial susceptibility testing methods. Determination of the MIC and MBC is needed to confirm antibacterial activity, while phytochemical characterization and quantitative determination of marker compounds are required to identify constituents that may contribute to the observed activity. Antibiofilm assays, cytotoxicity testing in skin cells, extract or formulation stability studies, and subsequent evaluation in in vivo models are also warranted before these extracts can be considered for further development as anti-acne preparations.

4. CONCLUSION

The 96% ethanol and ethyl acetate extracts of binahong leaves exhibited in vitro antibacterial activity against Cutibacterium acnes, with inhibition zone diameters increasing as the extract concentration increased. Both solvent type and extract concentration significantly affected the inhibition zone diameter, whereas their interaction did not have a significant effect. The ethyl acetate extract showed greater antibacterial activity than the 96% ethanol extract, particularly at concentrations of 4% and 6%, with the highest activity observed at 6%. These findings provide preliminary evidence for the potential of binahong leaf ethyl acetate extract as an antibacterial candidate against *C. acnes*; however, they should not be interpreted as evidence of clinical efficacy against acne vulgaris. Further studies are warranted, including determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), use of standardized C. acnes culture conditions, strain identification, chemical standardization of the extracts, and evaluation of safety and antibiofilm activity.

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