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Original Article

Evaluation of Antibacterial Activity of Ethanolic Green Spinach (*Amaranthus viridis*) Leaf Extract Against *Propionibacterium acnes*

Evaluasi Aktivitas Antibakteri Ekstrak Daun Bayam Hijau (*Amaranthus viridis*) dalam Etanol Terhadap *Propionibacterium Acnes*

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Abstract

Acne is a common chronic skin disorder among adolescents in Indonesia, with a prevalence of approximately 80–85%, particularly among individuals aged 15–18 years. One of the main microorganisms involved in acne pathogenesis is *Propionibacterium acnes*. This study aimed to evaluate the antibacterial activity of the ethanolic extract of green spinach (*Amaranthus viridis*) leaves against *P. acnes* and to identify its phytochemical constituents. This experimental study employed the agar well diffusion method. The extract was prepared using the maceration technique with 96% ethanol as the solvent. Phytochemical screening was conducted to qualitatively identify secondary metabolites, while antibacterial activity was assessed at extract concentrations of 50%, 60%, and 70%, with clindamycin as a positive control and sterile distilled water as a negative control. The phytochemical screening results showed the presence of flavonoids, tannins, and saponins in the ethanolic extract. However, the antibacterial evaluation demonstrated that the extract did not produce inhibition zones against *P. acnes* at any of the tested concentrations. In contrast, clindamycin exhibited a clear inhibition zone with a diameter of 19.25 mm, while the negative control showed no inhibitory effect. These findings indicate that, under the experimental conditions applied in this study, the ethanolic extract of green spinach leaves does not exhibit antibacterial activity against *P. acnes*. The results highlight the importance of extraction parameters and phytochemical characteristics in determining antibacterial effectiveness and suggest that further studies using alternative extraction methods, higher concentrations, or different biological activities, such as antioxidant assays, are warranted.

Abstrak

Jerawat merupakan salah satu gangguan kulit kronis yang umum terjadi pada remaja di Indonesia, dengan prevalensi sekitar 80–85%, terutama pada kelompok usia 15–18 tahun. Salah satu mikroorganisme utama yang berperan dalam patogenesis jerawat adalah Propionibacterium acnes. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri ekstrak etanol daun bayam hijau (Amaranthus viridis) terhadap P. acnes serta mengidentifikasi kandungan fitokimianya. Penelitian ini merupakan penelitian eksperimental dengan menggunakan metode difusi sumuran pada media agar. Ekstrak daun bayam hijau diperoleh melalui metode maserasi menggunakan pelarut etanol 96%. Skrining fitokimia dilakukan untuk mengidentifikasi secara kualitatif metabolit sekunder, sedangkan uji aktivitas antibakteri dilakukan pada konsentrasi ekstrak 50%, 60%, dan 70%, dengan klindamisin sebagai kontrol positif dan akuades steril sebagai kontrol negatif. Hasil skrining fitokimia menunjukkan bahwa ekstrak etanol daun bayam hijau mengandung senyawa flavonoid, tanin, dan saponin. Namun, hasil uji aktivitas antibakteri menunjukkan bahwa ekstrak tersebut tidak menghasilkan zona hambat terhadap P. acnes pada seluruh konsentrasi yang diuji. Sebaliknya, klindamisin sebagai kontrol positif menunjukkan zona hambat yang jelas dengan diameter 19,25 mm, sedangkan kontrol negatif tidak menunjukkan adanya daya hambat. Hasil penelitian ini menunjukkan bahwa, pada kondisi percobaan yang digunakan, ekstrak etanol daun bayam hijau tidak memiliki aktivitas antibakteri terhadap P. acnes. Temuan ini menegaskan pentingnya parameter ekstraksi dan karakteristik fitokimia dalam menentukan efektivitas antibakteri serta menunjukkan perlunya penelitian lanjutan dengan metode ekstraksi yang berbeda, konsentrasi yang lebih tinggi, atau pengujian aktivitas biologis lainnya, seperti uji aktivitas antioksidan.

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INTRODUCTION

Acne is one of the most common chronic skin disorders affecting adolescents and young adults, with a prevalence in Indonesia reaching approximately 80–85%, particularly among individuals aged 15–18 years. The high incidence of acne makes this condition a significant health problem, not only from a clinical perspective but also due to its psychological and social impacts on affected individuals. Pathophysiologically, acne is closely associated with sebum hypersecretion, follicular hyperkeratinization, microbial activity, and inflammatory responses. One of the main microorganisms involved in the pathogenesis of acne is Propionibacterium acnes, an anaerobic Gram-positive bacterium that produces lipase enzymes. These enzymes hydrolyze triglycerides into free fatty acids, which can trigger inflammation and stimulate the release of proinflammatory cytokines, thereby exacerbating acne severity[1].

Acne is generally triggered by hypersensitivity of the sebaceous glands to androgen hormones, leading to increased sebum production and the creation of an environment favorable for the growth of P. acnes. This bacterial activity subsequently aggravates the inflammatory processes occurring within the pilosebaceous unit. In addition to hormonal factors, acne severity is also influenced by other factors, including the use of certain medications such as lithium, steroids, and anticonvulsants, excessive sun exposure, the use of occlusive clothing, hormonal imbalances, and genetic predisposition. Clinically, acne is pleomorphic, presenting with a wide range of manifestations, from non-inflammatory comedones to inflammatory lesions such as papules and pustules. Bacterial activity plays a crucial role in worsening the clinical presentation of acne[2].

Acne management commonly involves the use of topical and systemic antibiotics, such as clindamycin and erythromycin, as well as chemical agents like benzoyl peroxide. Although these therapies are effective, long-term use may lead to adverse effects, including skin irritation and an increased risk of bacterial resistance. Antibiotic resistance has become a serious global health concern, including in acne therapy, highlighting the need for safer alternative treatments with minimal side effects. One promising approach is the utilization of natural products with antibacterial activity. Green spinach (Amaranthus viridis) is a plant with considerable potential to be developed as a natural antibacterial agent. This plant is known to contain various bioactive compounds, including flavonoids, phenolic compounds, saponins, and tannins. The tannin and flavonoid contents of green spinach have been reported to exhibit antibacterial activity through mechanisms involving the inhibition of microbial growth. In addition, A. viridis contains ribosome-inactivating proteins and beta-carotene and exhibits antiviral, antibacterial, antiproliferative, and antifungal properties. Traditionally, spinach has been used to treat various health conditions, including pain and fever, due to its analgesic and antipyretic effects[3]. Several previous studies have reported that spinach extracts possess antibacterial activity against various Gram-positive and Gram-negative bacteria. However, based on the existing literature, studies that specifically evaluate the antibacterial activity of ethanolic extracts of green spinach leaves against Propionibacterium acnes remain limited, and the available findings show inconsistent effectiveness[4].

Moreover, investigations into the effect of different extract concentrations on the inhibitory activity against P. acnes are still scarce. This research gap indicates the need for further studies to explore the antibacterial potential of ethanolic extracts of green spinach leaves against acne-causing bacteria. Therefore, this study aims to evaluate the

antibacterial activity of the ethanolic extract of green spinach leaves (*Amaranthus viridis*) against the growth of Propionibacterium acnes. The results of this study are expected to provide scientific evidence supporting the development of safe and effective natural-based alternative therapies for acne, with the potential to reduce dependence on synthetic antibiotics.

METHODS

This study was an experimental research conducted at two different locations. The preparation of simplisia, ethanol-free testing, and phytochemical screening were carried out at the Laboratory of STIKES Darul Azhar Batulicin. Meanwhile, the antibacterial activity assay was conducted at the Public Health Laboratory of Tanah Bumbu Regency. This study aimed to evaluate the antibacterial activity of the ethanol extract of green spinach leaves (*Amaranthus viridis*) against the growth of *Propionibacterium acnes* using the agar well diffusion method. The extract was tested at three different concentrations, namely 50%, 60%, and 70%. Sterile distilled water was used as the negative control, while clindamycin served as the positive control.

Instruments

The main instruments used in this study included an analytical balance, hot plate, vortex mixer, autoclave, laminar air flow cabinet, light microscope, and magnetic stirrer. Routine laboratory equipment was not described in detail.

Materials

The materials used in this study consisted of 96% ethanol, nutrient agar, Mueller Hinton Agar (MHA), 0.9% NaCl solution, sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), 10% ferric chloride ($FeCl_3$) solution, 10% gelatin solution, clindamycin 300 mg, sterile distilled water, green spinach leaves (*Amaranthus viridis*), and the test bacterium *Propionibacterium acnes*.

Research Procedures

Collection of Plant Material

Fresh green spinach leaves were obtained from Ampera Market, Tungkaran Street, Batulicin, Tanah Bumbu Regency. The spinach plants were cultivated by local farmers and used in fresh condition.

Preparation of Spinach Leaf Extract

A total of 250 g of powdered spinach leaf simplisia was weighed and placed into a glass container. Extraction was performed using the maceration method with 96% ethanol as the solvent at a ratio of 1:10, corresponding to 250 g of simplisia powder in 2.5 L of solvent. The use of 96% ethanol was based on its effectiveness in extracting phenolic, flavonoid, and tannin compounds. Maceration was carried out for three days in a dark place with stirring every 12 hours. After completion, the mixture was filtered to obtain the filtrate, which was then evaporated using a water bath to produce a concentrated extract.

Phytochemical Screening

The concentrated extract was subjected to phytochemical screening to identify the presence of flavonoids, phenolic compounds, saponins, and tannins.

Ethanol-Free Test

The ethanol-free test was conducted qualitatively by adding concentrated sulfuric acid (H_2SO_4) and potassium dichromate solution to the extract. A color change from orange to bluish-green indicated the presence of ethanol[5].

Sterilization of Instruments and Media

Glassware and culture media were sterilized using an autoclave at 121°C for 15 minutes. Inoculating loops and forceps were sterilized by flaming over a spirit lamp prior to use.

Preparation of Mueller Hinton Agar

A total of 8.5 g of Mueller Hinton Agar was dissolved in 250 mL of distilled water and heated until homogeneous. The medium was sterilized in an autoclave at 121°C for 15 minutes, then aseptically poured into Petri dishes and allowed to solidify before use.

Gram Staining of *Propionibacterium acnes*

Bacterial cells were collected using a sterile inoculating loop, mixed with distilled water on a glass slide, air-dried, and heat-fixed. Gram staining was performed using crystal violet, Lugol's iodine, alcohol, and carbol fuchsin. The stained slides were observed under a light microscope at 100× magnification.

Bacterial Rejuvenation

The test bacterium *P. acnes* was rejuvenated by streaking 1–2 loopfuls of culture onto the surface of TSA medium in a zig-zag pattern, followed by incubation at 37°C for 24 hours to obtain fresh bacterial colonies[6].

Preparation of Bacterial Suspension

Fresh colonies of *P. acnes* were collected using a sterile inoculating loop and suspended in 10 mL of sterile distilled water until the turbidity was equivalent to the 0.5 McFarland standard (Gerung et al., 2021).

Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion method. A total of 200 µL of bacterial suspension was evenly spread on the surface of MHA medium. Wells with a diameter of 6 mm were made in the agar, and 50 µL of extract at each concentration was added into the wells. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones was measured using a caliper[7].

Interpretation of Results

The inhibition zone diameters were categorized according to the criteria of David and Stout (1971): weak (<5 mm), moderate (5–10 mm), strong (11–20 mm), and very strong (>20 mm)[8].

Data Analysis

The data obtained were analyzed using SPSS software. If the data were normally distributed and homogeneous, One-Way ANOVA was applied. If these assumptions were not met, the non-parametric Kruskal–Wallis test was used.

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RESULT AND DISCUSSION

This section presents the results of the study along with their interpretation and discussion in relation to previous research. The findings are discussed to highlight their significance, similarities, and differences compared to earlier studies. This study aimed to evaluate the antibacterial activity of the ethanol extract of green spinach leaves (*Amaranthus viridis*) against the growth of *Propionibacterium acnes*. The extract was obtained using the maceration method with concentration variations of 50%, 60%, and 70%.

1. Extract Yield

The extraction of green spinach leaves was carried out using the maceration method with 96% ethanol as the solvent. A total of 2 kg of fresh spinach leaves were dried to obtain 250 g of dry powder, which was then macerated for five days. The extraction process yielded 25.2344 g of thick extract, corresponding to a percentage yield of 10.09%. This yield indicates that ethanol was able to extract a considerable amount of soluble compounds from the spinach leaves, particularly polar and semi-polar secondary metabolites.

2. Phytochemical Screening Results

Phytochemical screening was conducted to qualitatively identify the presence of bioactive compounds in the ethanol extract of green spinach leaves. The results of the screening are presented in Table 1.

Table 1. Phytochemical Screening Results of Ethanol Extract of Green Spinach Leaves

No	Class of Compound	Result	Indicator
1	Flavonoids	+	Orange-red color
2	Phenolics	–	No color change
3	Saponins	+	Foam formation
4	Tannins	+	Greenish-black color

Information:

(+) indicates the presence of the compound; (−) indicates the absence of the compound. Flavonoids were identified by the formation of an orange-red color, saponins by stable foam formation, and tannins by the appearance of a greenish-black coloration after reagent addition.

The phytochemical screening results indicated the presence of flavonoids, saponins, and tannins in the ethanol extract of green spinach leaves, while phenolic compounds were not detected. Although these secondary metabolites have been reported to exhibit antibacterial properties in other plant species, their presence in this extract did not result in measurable antibacterial activity against Propionibacterium acnes under the experimental conditions applied. This suggests that the concentration, composition, or bioavailability of these compounds may not have been sufficient to exert antibacterial effects. Previous studies have reported antibacterial activity of saponins against certain Gram-positive bacteria, such as Staphylococcus aureus[9]. However, such effects were not observed in the present study, indicating that differences in plant source, extraction method, and target microorganisms may influence antibacterial outcomes.

3. Gram Staining of Propionibacterium acnes

Microscopic observation of Gram-stained preparations revealed that Propionibacterium acnes appeared as purple-colored rod-shaped (bacilli) bacteria, indicating that it is a Gram-positive bacterium. The retention of the purple color is attributed to the thick peptidoglycan layer in the cell wall of Gram-positive bacteria, which enables the crystal violet–iodine complex to remain intact despite alcohol decolorization.

4. Inhibition Zone Test at 50%, 60%, and 70% Concentrations

The antibacterial activity of the ethanol extract of green spinach leaves against P. acnes was evaluated using the agar well diffusion method. The inhibition zone diameters are presented in Table 2.

Table 2. Inhibition Zone Diameter of Propionibacterium acnes

Treatment	Inhibition Zone (mm)	Category
Positive control (Clindamycin)	19.2	Strong
Negative control	0	No inhibition
Extract 50%	0	No inhibition
Extract 60%	0	No inhibition
Extract 70%	0	No inhibition

Information:

The positive control was clindamycin, while the negative control was sterile distilled water. Inhibition zone diameter was measured in millimeters (mm). Antibacterial activity was classified according to David and Stout (1971) as follows: weak (<5 mm), moderate (5–10 mm), strong (11–20 mm), and very strong (>20 mm).

The results showed that no inhibition zones were formed around the wells containing the ethanol extract at concentrations of 50%, 60%, and 70%. In contrast, the positive control (clindamycin) produced a strong inhibition zone, while the negative control showed no antibacterial activity. These findings indicate that, under the experimental conditions applied in this study, the ethanol extract of green spinach leaves did not exhibit significant antibacterial activity against P. acnes. The absence of inhibition zones may be attributed to the strong defense mechanisms of P. acnes. As a Gram-positive anaerobic bacterium, P. acnes possesses a thick peptidoglycan layer that provides protection against osmotic stress and antimicrobial agents. Additionally, this bacterium is capable of forming biofilms, which act as a protective barrier and enhance resistance to antibiotics and host immune responses. P. acnes also produces lipase enzymes that hydrolyze triglycerides in sebum into free fatty acids, contributing to its survival and pathogenicity[10].

Another factor that may have influenced the results is the choice of extraction solvent. Although 96% ethanol was selected due to its universal solvent properties, low toxicity, and ability to dissolve a wide range of organic compounds, several studies have reported that methanol may extract higher levels of certain bioactive compounds depending on the type of secondary metabolites present[12]. Differences in solvent polarity can significantly affect the composition and concentration of extracted bioactive compounds[11].

The absence of inhibition zones across all tested concentrations indicates that this study was unable to determine the optimal concentration of green spinach leaf extract for inhibiting P. acnes. These findings are consistent with previous research using red spinach leaf extract at a concentration of 25%, which also did not produce inhibition zones[13] . Further studies are therefore required using alternative extraction methods, higher extract concentrations, or different test organisms to comprehensively evaluate the antibacterial potential of spinach leaf extracts. Statistical analysis was performed using the Kruskal–Wallis test as an alternative to One-Way ANOVA due to non-normal data distribution or lack of homogeneity of variance. The results showed a significance value (p) of 0.406 ($p > 0.05$), indicating no statistically significant differences among the treatment groups. Therefore, the null hypothesis (H_o) was accepted, and the alternative hypothesis (H_1) was rejected. Overall, the ethanol extract of green spinach leaves (Amaranthus viridis) did not demonstrate antibacterial activity against Propionibacterium acnes at the tested concentrations.

LIMITATION

This study has several limitations that should be considered when interpreting the findings. First, the antibacterial activity of the ethanol extract of green spinach leaves (*Amaranthus viridis*) was evaluated only using the well diffusion method, which may be less sensitive for detecting antibacterial effects of extracts with limited diffusion capacity in solid media. Second, the concentrations tested were limited to 50%, 60%, and 70%, which may not have been sufficient to demonstrate inhibitory activity against *Propionibacterium acnes*. In addition, the extraction process was conducted using a single solvent (96% ethanol), which may not have optimally extracted all bioactive compounds

responsible for antibacterial activity. This study also focused on a single bacterial species, thereby limiting the generalizability of the results to other acne-associated microorganisms. Future research is recommended to employ alternative extraction solvents, higher extract concentrations, different antibacterial testing methods, and a broader range of bacterial strains to more comprehensively evaluate the antibacterial potential of *Amaranthus viridis*.

CONCLUSION

This study demonstrates that the ethanol extract of green spinach leaves (*Amaranthus viridis*) did not exhibit antibacterial activity against *Propionibacterium acnes* at the tested concentrations of 50%, 60%, and 70% under the experimental conditions employed. These findings indicate that, although green spinach leaves contain bioactive compounds with reported antibacterial potential, their effectiveness against *P. acnes* may be influenced by factors such as extraction method, solvent polarity, concentration, and bacterial characteristics, including cell wall structure and biofilm-forming ability. The absence of inhibitory activity highlights the importance of optimizing extraction techniques and testing higher concentrations or alternative solvents to enhance the availability of active constituents. Furthermore, the results underscore the need for complementary antibacterial assays and broader microbial targets to comprehensively evaluate the therapeutic potential of plant-based extracts. Future studies are recommended to explore different extraction approaches, formulation strategies, or synergistic combinations with other natural agents to support the development of effective and sustainable herbal-based anti-acne therapies.

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AUTHOR CONTRIBUTION STATEMENT (Calibri, 12 pt)

ADR: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing Original Draft Preparation.

ARD: Supervision, Validation, Writing Reviewing and Editing.

AZR: Visualization, Software, Formal analysis.

All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

CONFLICT OF INTEREST DECLARATION (Calibri, 12 pt)

The authors declare no conflicts of interest associated with this study.

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