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Effectiveness test of eco enzyme antibiotics towards the growth of *Cutibacterium acnes* bacteria in vitro

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ABSTRACT

Acne vulgaris is a common skin infection in Indonesia, especially among adolescents, triggered by tropical climate, pollution, and *Cutibacterium acnes* bacteria. Long-term use of antibiotics poses risks of side effects and bacterial resistance, leading to the need for natural alternatives such as eco enzyme, which contains antimicrobial compounds, acetic acid, and alcohol. This study aims to determine the effectiveness of eco enzyme against *C. acnes* growth. The method used was an experimental post-test only design with four eco enzyme concentrations (25%, 50%, 75%, and 100%) a positive control using clindamycin, and a negative control using DMSO, with an antibacterial testing period of five days. The test was conducted with five repetitions. Inhibition zone diameters were measured using a caliper and analyzed with ANOVA and Tukey's post hoc test. Results showed antibacterial activity with an inhibition zone of 2.2 mm at a concentration of 25%, 4.9 mm at 50%, 6.6 mm at 75%, and 9.99 mm at 100% concentration, and effectiveness increased with concentration. It is concluded that eco enzyme exhibits weak to moderate antibacterial activity against *C. acnes*, possibly influenced by the limited phytochemical content and the presence of bacterial biofilms.

INTRODUCTION

Infection is one of the most common health problems, especially in tropical countries such as Indonesia. Humid and dusty environmental conditions, along with pollution from transportation and inadequate sanitation, increase the exposure of communities to pathogenic microorganisms. These conditions facilitate the proliferation of bacteria, viruses, fungi, and protozoa that may contribute to various infectious disease (Pariury, 2021). One of the most prevalent skin infections in Indonesia is acne vulgaris, commonly known as acne, which frequently affects adolescents and young adults.

Acne vulgaris is characterized by the presence of comedones, papules, pustules, nodules, and erythema. The hot and humid Indonesian climate may contribute to conditions that are favourable for the colonization and growth of acne-related bacteria. The prevalence of acne among Indonesian adolescents is notably high, reaching 80–90% in recent years

(Ollyvia et al., 2021). According to the Global Burden of Disease (GBD) data, acne vulgaris affects approximately 85% of individuals aged 12–25 years (Hazarika, 2021).

Acne is generally caused by excessive sebum production that clogs skin pores, influenced by hormonal factors, stress, pollution, cosmetic use, and bacterial infection by *C. acnes*. C. acnes produces lipase enzymes that hydrolyze sebum triglycerides into free fatty acids, which then trigger local irritation. These free fatty acids activate TLR-2 signaling pathways and promote biofilm formation, leading to enhanced inflammation within the pilosebaceous unit (Ramadhani et al., 2022). This gram-positive anaerobic bacterium resides in human hair follicles and thrives in oxygen-deprived environments, particularly when pores are blocked by sebum, leading to inflammation (Munandar Nasution et al., 2022). Besides the face, *C. acnes* can also be found on other parts of the body such as the back, chest, and neck. The bacterium may also spread through everyday objects like makeup tools, especially when cosmetics contain comedogenic and acnegenic ingredients such as lanolin, alcohol, dyes, and preservatives (Fadilah et al., 2024).

Treatment of acne vulgaris typically involves topical and systemic agents such as benzoyl peroxide, retinoid, salicylic acid, isotretinoin, and antibiotics including clindamycin, erythromycin, and tetracycline. However, improper and prolonged use of antibiotics can lead to bacterial resistance, reduced therapeutic effectiveness, and adverse effects such as skin irritation, dryness, and hypersensitivity reactions (Wu et al., 2021). Moreover, other bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* are also involved in acne pathogenesis and may exhibit cross-resistance to antibiotics (Legiawati et al., 2023).

Under these circumstances, individuals have begun to seek alternative treatments that are safer, more effective, and environmentally friendly. One increasingly popular approach is the use of natural ingredients derived from plants or animals, which are known to contain vitamin C and various bioactive compounds such as alkaloids, phenolics, and tannins. These compounds have potential as alternative therapies for the treatment of acne vulgaris. According to the World Health Organization (WHO), approximately 80% of the population in developing countries still relies on plant-based traditional medicine, with more than 20,000 plants species recorded as potential medicinal sources (Vaou et al., 2021).

One of the natural product that has recently gained attention in the health field is eco enzyme, a fermented solution derived from organic waste such as fruit peels, which are rich in flavonoids and antimicrobial compounds. Eco enzyme contains alcohol, acetic acid, and lactic acid, which synergistically act as antimicrobial, antifungal, and insecticidal agents. The presence of organic acids, enzymes, and alcohol in eco enzyme formulations may disrupt bacterial cell membranes and interfere with microbial metabolism. These properties suggest a potential inhibitory effect on anaerobic bacteria such as *Cutibacterium acnes* (Vama and Cherekar, 2020). The previous study by Aisyah, Rizkya, and Riska demonstrated antibacterial activity of eco-enzyme made from pineapple peel against Staphylococcus aureus and Cutibacterium acnes (Ramadhani et al., 2022).

However, evidence regarding the antibacterial activity of eco enzyme specifically against *C. acnes* remains limited, and no standardized laboratory evaluation has yet been reported. This study aims to evaluate the antibacterial potential of eco enzyme against *Cutibacterium acnes*, one of the main causative agents of acne vulgaris.

RESEARCH METHODS

The study was conduced at the Microbiology Laboratory of Universitas Islam Sumatera Utara in January 2025. In this study, a quantitative experimental approach was applied using a post-test only group design. The experiment comprised six treatment groups, each performed in five replications, as follows:

a. Positive control (Clindamycin)
b. Negative control (DMSO)
c. Treatment group 1 (eco enzyme at 25% concentration)
d. Treatment group 2 (eco enzyme at 50% concentration)
e. Treatment group 3 (eco enzyme at 75% concentration)
f. Treatment group 4 (eco enzyme at 100% concentration)

The instruments used in this study included petri dishes, petri dish racks, inoculating loops, vernier calipers, paper discs, measuring cylinders, Erlenmeyer flasks, drop pipettes, micropipettes, incubators, analytical balances, spirit burners, test tubes, autoclaves, test tube holders, and hot plates. The materials used comprised *Cutibacterium acnes* isolates, eco enzyme solutions at concentrations of 25%, 50%, 75%, and 100%, Mueller Hinton Agar (MHA) medium, 0.9% NaCl solution, 96% ethanol, DMSO, clindamycin, and distilled water. The sample size in this study was determined using the Federer formula, as follows:

$(n-1)(t-1) \geq 15$
$(n-1)(6-1) \geq 15$
$(n-1)(5) \geq 15$
$5n-5 \geq 15$
$5n \geq 20$
$n \geq 4$

A minimum of four replications per group was required.

Preparation

Preparation of equipment and materials for antibacterial testing began with sterilizing the instruments using a hot air oven at 170°C for one hour. The culture media were sterilized using an autoclave at 121°C for 15–20 minutes, while the inoculating loop and forceps were sterilized by flaming over a Bunsen burner.

Media preparation

The Mueller Hinton Agar (MHA) medium was prepared by weighing 38 grams of MHA powder according to the composition stated on the package. The medium was then dissolved in 1 liter of distilled water and sterilized using an autoclave at 121°C for 15–20 minutes. After sterilization, the MHA medium was poured into sterile Petri dishes and allowed to solidify at room temperature. The solidified media were stored at 4°C until use. Prior to the experiment, the media were examined to ensure they were free from contamination.

Bacterial rejuvenation

A single colony of *Cutibacterium acnes* was isolated using a sterile inoculating loop that had been flamed over a Bunsen burner. The colony was then transferred onto MHA medium in a Petri dish and incubated at 37°C for 24 hours.

Phytochemical screening

1. Identification of Flavonoid (Shinoda Test)

A total of 1 mL of the eco enzyme was reacted with 2 mg of magnesium (Mg) powder and 3 drops of 37% HCl. The presence of flavonoids was indicated by a colour change of the sample to red, yellow, or orange.

2. Identification of Alkaloid (Mayer's Test)

A total of 1 mL of the eco enzyme was reacted with Mayer's reagent. A positive result was indicated by the formation of a white to yellowish precipitate

3. Identification of Tannin (Ferric Chloride Test)

A total of 1 mL of the eco enzyme was reacted with 5 drops of 1% $FeCl_3$ reagent. A positive result was indicated by a color change to dark blue or dark green.

4. Identification of Saponin (Froth Test)

A total of 1 mL of the eco enzyme was placed in a test tube and mixed with 10 drops of distilled water. A positive result was indicated by the formation of stable foam that persisted for 30 minutes and did not disappear after the addition of 1 drop of 2N HCl.

Preparation of eco enzyme concentration

Eco enzyme was prepared using a fermentation method. The substrates consisted of watermelon (*Citrullus lanatus*), citrus (*Citrus* sp.), papaya (*Carica papaya*), banana (*Musa* sp.) and dragon fruit (*Hylocereus polyrhizus*) peels. A total of 3 kg of mixed fruit peels were washed, cut into small pieces, and combined in a single container. 1 kg molasses was then added, followed by 10 liters of water into the 25 liters fermentation container. The mixture was allowed to ferment for 100 days. After the fermentation period, the eco enzyme was deemed ready for use and subsequently separated from the sediment deposited at the bottom of the container. Then the eco enzyme was evaluated for its antibacterial activity at concentration of 25%, 50%, 75%, and 100% (Figure 1.) with dimethyl sulfoxide (DMSO) utilized as the diluent.

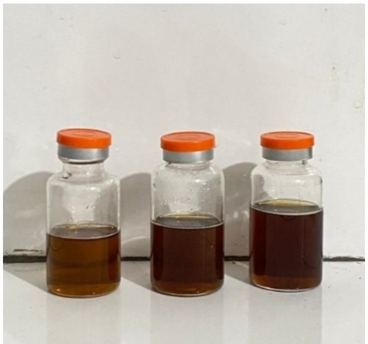

Figure 1. Eco Enzyme with Concentration 25%, 50% and 75%

Antibacterial sensitivity test

The antimicrobial sensitivity test was conducted by preparing agar plates and Petri dishes containing colonies of *Cutibacterium acnes*. Paper discs were first sterilized in an oven at 70°C for 15 minutes, then soaked in 1 mL of the test solution for 15 minutes. Bacterial colonies were inoculated into liquid medium in test tubes and incubated at 35–37°C for 2–5 hours until the turbidity matched the 0.5 McFarland standard. The bacterial suspension was then evenly spread onto the surface of Mueller Hinton Agar (MHA) using a sterile cotton swab and left to stand for 3–5 minutes. Subsequently, the discs containing the test samples were placed on the agar surface using sterile forceps with gentle pressure. The plates were incubated at 37°C for 18–24 hours, after which the clear inhibition zones around the discs were measured using a vernier caliper and recorded in millimeters.

Data analysis

The research results were measured using a vernier caliper, and data analysis was performed using one-way ANOVA followed by Tukey's post hoc test with GraphPad Prism version 10. The statistical tests were conducted to determine the most effective concentration of the eco enzyme in inhibiting the growth of *Cutibacterium acnes*.

This study obtained ethical approval from the Health Research Ethics Committee (HREC) of the Faculty of Medicine, Universitas Islam Sumatera Utara (UISU) with approval number No.02/EC/KEPK.UISU/V/2025.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the eco enzyme was carried out to identify the metabolite compounds present in the solution that may contribute to its antibacterial activity. The results of the phytochemical screening indicated that the tested eco enzyme contained terpenoids, saponins, and tannins (Table 1.)

Table 1. The Result of Phytochemical Screening

Eco enzyme		
Secondary metabolites	**Reagent**	**Results**
Alkaloid	Liebermann-Bouchard	-
	Mayer's	-
Flavonoid	$FeCl_3$	-
	MgHCl	-
Terpenoid	Liebermann-Bouchard	+
	Salkowsky	-
Steroid	Liebermann-Bouchard	-
	Salkowsky	-
Saponin	Aquadest	+
Tannin	$FeCl_3$	+

Notes:
(+) : Presence of secondary metabolites
(-) : Absence of secondary metabolites

Antibacterial activity

The results of the antibacterial effectiveness test of the eco enzyme derived from papaya peel (*Carica papaya*), banana peel (*Musa* sp.), watermelon rind (*Citrullus lanatus*), dragon fruit peel (*Hylocereus polyrhizus*), and orange peel (*Citrus* sp.) against the growth of *Cutibacterium acnes* demonstrated bacterial inhibition activity. Based on the disc diffusion assay, inhibition zones were formed around the discs treated with eco enzyme at concentrations of 25%, 50%, 75%, and 100% (Table 2.)

Table 2. Results of Antibacterial Inhibition Measurement Against *C. acnes*

Replication	Diameter of bacteria growth inhibition against *C. acnes* (in mm)					
	Eco enzyme concentration				Positive control	Negative control
	25%	50%	75%	100%	Clindamycin	DMSO
U1	2.1	4.2	6.3	7.8	27.1	0
U2	2	4.2	6.2	7.9	26	0
U3	2.1	4.4	6	9.3	27.9	0
U4	2.2	4.9	6.6	9.9	26.8	0
U5	2.2	4.9	6.5	9.9	26.1	0

Table 3. Results of the Shapiro-Wilk Normality Test and One Way ANOVA

Groups	Shapiro-Wilk	One-way ANOVA
Eco enzyme 25%	P = 0.314	
Eco enzyme 50%	P = 0.069	
Eco enzyme 75%	P = 0.898	P = <0.0001
Eco enzyme 100%	P = 0.099	
Clindamycin (+)	P = 0.609	

The data analysis revealed significant differences in the antibacterial effectiveness of the eco enzyme at concentrations of 25%, 50%, 75%, and 100% (Table 2.) compared to the positive control (clindamycin) and the negative control (DMSO) . The largest inhibition zone was observed at the 100% concentration; however, it was still significantly smaller than that of clindamycin, which exhibited high sensitivity with a diameter greater than 20 mm according to EUCAST standards. At concentrations of 25% and 50%, inhibition zones were present but very weak (≤5 mm), whereas concentrations of 75% and 100% produced moderate inhibition (5–10 mm) based on the criteria established by Davis and Stout (Table 4.). ANOVA revealed statistically significant differences among treatment groups ($p < 0.05$) (Table 3.). Tukey's post hoc test (Figure 2.) showed that the 75% and 100% concentrations differed significantly from the 25% and 50% groups.

Table 4. Bacterial Growth Inhibition Categories (Davis and Stout)

No	Category	Clear zone diameter (mm)
1	Very strong	≥ 20
2	Strong	10-20
3	Moderate	5-10
4	Weak	≤ 5

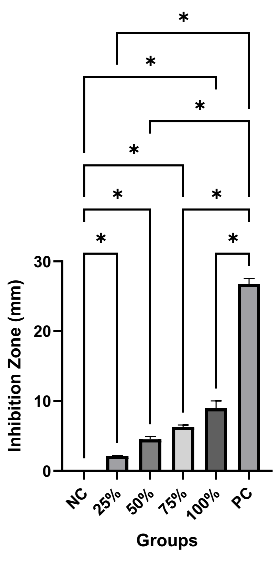

Figure 2. Graph of the Post-Hoc ANOVA Results

Based on the graph of the Post-hoc ANOVA test results, the antibacterial effectiveness of the test samples increased with each rise in concentration. The 25% concentration group showed the smallest inhibition zone, although it was still significantly larger than the negative group. At the 50% concentration, the inhibition zone began to increase gradually, continuing to rise at the 75% concentration, and reaching its highest value at the full concentration of 100%. The graph indicates that increasing the concentration results in a progressive enchantment of antibacterial activity.

Eco enzyme is a fermented liquid derived from organic waste such as vegetables and fruit peels, containing various phytochemical compounds and natural enzymes. Ingredients such as citrus, pineapple, papaya, banana, and watermelon peels provide vitamin C, flavonoids, limonene, bromelain, papain, and lipase as bioactive components (Gumilar, 2023). During fermentation, microorganisms including *Aspergillus niger*, *Saccharomyces cerevisiae*, *Acetobacter*, *Lactobacillus*, and *Pseudomonas* break down the organic substrates and produce important metabolites, including lactic acid, acetic acid, ethanol, as well as amylase and protease enzymes. Both homofermentative and heterofermentative processes enrich the composition of the eco enzyme through the formation of organic acids and additional bioactive compounds. The synergy between the phytochemicals in the initial raw materials and the metabolites generated during fermentation leads to effective biological activity, such as pH reduction, microbial membrane disruption, degradation of cell wall proteins, and inhibition of pathogenic bacterial growth. Thus, the effectiveness of eco enzyme results from the integrated contribution of its chemical constituents and the biochemical transformations occurring throughout the fermentation process (Putu et al., 2023). During fermentation, microorganisms break down organic substrates into active metabolites such as acetic acid, ethanol, and CO_2. Acetic acid plays a dominant antibacterial role by penetrating the bacterial cell membrane through a pH gradient, disrupting internal metabolic processes, and inducing osmotic lysis due to osmotic pressure. As the fermentation duration increases, the concentration of acetic acid also rises, thereby enhancing the antimicrobial activity of the eco-enzyme (Permatananda et al., 2023).

The antibacterial activity of the eco enzyme is influenced not only by its concentration but also by the presence of phytochemical compounds contained within it. The detected compounds—terpenoids, saponins, and tannins—exhibit different mechanisms of bacterial inhibition. Although these secondary metabolites exert known antimicrobial effects, their concentrations in the eco enzyme were likely insufficient to produce strong inhibition against *C. acnes*. Terpenoids are lipophilic in nature, enabling them to disrupt cell membranes, interfere with bacterial communication systems, and cause the release of intracellular proteins and enzymes (W. Huang et al., 2022). Saponins act by altering cell membrane structure, compromising its integrity, and enhancing antibiotic effectiveness synergistically by increasing permeability and inhibiting biofilm formation (Li & Monje-Galvan, 2023). Meanwhile, tannins cause cell membrane contraction, which increases permeability and disrupts bacterial metabolism, ultimately leading to cell lysis (J. Huang et al., 2024). Nevertheless, the antibacterial activity of the eco enzyme remains in the weak-to-moderate category, presumably due to insufficient concentrations of active compounds to penetrate the protective biofilm produced by *C. acnes* (Aji et al., 2023).

The stability of the active compounds after the fermentation process may also affect antibacterial activity, resulting in inhibition zones that are less effective compared to the positive control (clindamycin). The reduced activity at lower concentrations may indicate partial degradation of volatile antimicrobial compounds during fermentation. Biologically, *C. acnes* is a facultative anaerobic Gram-positive bacterium that inhabits hair follicles and sebaceous glands, produces lipase enzymes that trigger inflammation, and forms biofilms that increase resistance to conventional therapies (Beig et al., 2024). Therefore, the presence of active components such as acetic acid, proteolytic enzymes, and secondary metabolites generated during fermentation in the eco enzyme has the potential to disrupt biofilm formation and suppress bacterial enzymatic activity. The limited inhibition zones observed may be attributable to the robust biofilm phenotype of *C. acnes*, which reduces susceptibility to surface-applied disc diffusion agents. This is evidenced by the formation of significant inhibition zones in the in vitro assay, although the overall effectiveness remains limited (Kim et al., 2024).

CONCLUSION

This study concluded that the eco enzyme formulated from a mixture of papaya peel (*Carica papaya*), banana peel (*Musa sp*.), watermelon rind (*Citrullus lanatus*), dragon fruit peel (*Hylocereus polyrhizus*), and orange peel (*Citrus* sp.) exhibited antibacterial activity against *Cutibacterium acnes*. The highest inhibitory effect was observed at a 100% concentration with an inhibition zone diameter of 9.99 mm (moderate category), followed by the 75% concentration with a diameter of 6.6 mm (moderate category), the 50% concentration with a diameter of 4.9 mm (weak category), and the 25% concentration with a diameter of 2.2 mm (weak category). Nonetheless, the antibacterial effect remained inferior to clindamycin, indicating that eco enzyme cannot yet serve as a standalone therapy and requires further optimization.

Author Contributions

This study on the antibacterial effectiveness of eco enzyme against *Propionibacterium acnes* was conducted through the collaboration of all listed authors. Each author made a significant contribution to various stages of the research. Conceptualization and research design were led by Farah Suhaila and Ramadhan Bestari, laboratory experiments and data collection were carried out by Farah Suhaila, data analysis and interpretation were performed by Farah Suhaila; validation and supervision were conducted by Dewi Yanti Handayani and Siti Kemala Sari visualization and initial manuscript drafting were completed by Farah Suhaila, while the review and editing of the manuscript were conducted jointly by Farah Suhaila, Ramadhan Bestari, Dewi Yanti Handayani, and Siti Kemala Sari. All authors have read and approved the final version of the manuscript and agree to take full responsibility for all aspects of this research, including the accuracy, integrity, and originality of its content.

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Conflicts Of Interest

The authors declare no conflict of interest. This research was conducted using the personal funds of the authors. No external funding was received, and no sponsor was involved in the design, execution, analysis, or publication of this study.

Abbreviations

The following list of abbreviations is included in this article. This list ensures clarity and ease of understanding for readers.

- ANOVA – Analysis of Variance
- C. Acnes – Cutibacterium acnes
- CO_2 – Carbon Dioxide
- DMSO – Dimethyl Sulfoxide
- EUCAST – European Committee on Antimicrobial Susceptibility Testing
- $FeCl_3$ – Ferric Chloride
- GBD – Global Burden of Disease
- g – Gram
- HCl – Hydrochloric Acid

- HREC – Health Research Ethics Committee
- kg – Kilogram
- L – Liter
- Mg – Magnesium
- mL – Milliliter
- mm – Millimeter
- MHA – Mueller–Hinton Agar
- N – Normality
- NaCl – Sodium Chloride
- NC – negative control
- pH – Potential of Hydrogen
- PC – positive control
- TLR-2 – Toll-Like Receptor 2
- WHO – World Health Organization
- °C – Degree Celsius

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