

Antibacterial Activity of Pineapple Peel (*Ananas comosus*) Eco-enzyme Against Acne Bacteria (*Staphylococcus aureus* and *Prapionibacterium acnes*)Aisyah Hadi Ramadani¹, Rizky Karima², Riska Surya Ningrum^{3*}¹Biology Study Program, Faculty of Science, Technology and Education, University of Muhammadiyah Lamongan, Jl. Plalangan, Plosowahyu, Lamongan, Indonesia²Chemistry Study Program, Faculty of Mathematics and Sciences, University of Pakuan, Jl. Pakuan, Tegal Lega, Bogor, Indonesia³Research Center for Biomaterial, National Research and Innovation Agency, Jl. Raya Bogor KM. 46 Cibinong, Bogor, Indonesia

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Abstract

Acne (acne vulgaris) is a skin disease caused by infection or inflammation of the pilosebaceous unit. *Staphylococcus aureus* and *Prapionibacterium acnes* was the main actor in the infection. Eco-enzyme that is made from pineapple peel has been stated that have inhibitory activity against gram-positive and negative bacteria, also applied as home industry soap. This study objective as scientific support that Eco-enzyme phytochemical compounds had the antibacterial activity to the acne bacteria. Eco-enzyme was composed of pineapple peel, brown sugar, and water with the ratio of 3:1:10 for 3 months fermentation time. Eco-enzyme was screened of its phytochemical compound and antibacterial activity against *S.aureus* and *P.acnes* by dilution with various concentration (1.5625%, 3, 125%, 6.25%, 12.5%, 25%, and 50% v/v) and various control. The minimum inhibition concentration (MIC) was then tested by diffusion method to determine the inhibition zone with 3 replication using the higher concentration (50%, 75%, 100% v/v). The result showed that eco-enzyme has a clear yellow colour, contains tannin and saponin, MIC of *S.aureus* and *P.acnes* bacteria is 50%. The dilution test of eco-enzyme gives the most effective concentration to inhibit *S.aureus* at eco-enzyme 100% (v/v), contrasted to *P. acnes* which didn't show the best inhibition concentration.

Keywords: Acne, antibacterial, eco-enzyme, pineapple peel, *S.aureus*, *P.acnes*

INTRODUCTION

Acne vulgaris is a skin problem that often occurs in teenagers. Acne is formed due to infection and inflammation of the pilosebaceous glands (oil glands) (Mohiuddin, 2019). The causes of infection and inflammation include genetics, environment, cosmetics, drugs, diet, stress, hormones (Ayudianti & Indramaya, 2014), skin hygiene, and bacterial infections (Kumar et al., 2016). Bacteria that causing acne are *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Prapionibacterium acnes* (Sitohang, Fathan, Effendi, & Wahid, 2019).

S. aureus is a gram-positive, coccus form bacteria that causes an abscess of pus at the skin layer (Kumar et al., 2016). *P. acnes* is also a gram-positive bacteria and bacilli (rod) form that causes infections in rich sebaceous glands, such as the skin on the face. *P.acnesa* normal skin flora whose numbers will

increase after the maturation of the sebaceous gland function (puberty ages).

Acne is a self-limiting disease, but it can reduce the self-confidence of the sufferer so that giving acne medication is a more promising option. Acne medications, both natural and synthetic, contain active compounds that can kill or inhibit the growth of acne-causing bacteria. Synthetic acne drugs that have been commercialized include minocycline, tetracycline, erythromycin, doxycycline, and clindamycin (Sitohang et al., 2019). Its synthetic antibacterial drugs (antibiotics) cause bacterial resistance, so people prefer the use of natural antibiotics. Natural acne medications are generally made from plant or animal extracts that contain vitamin C or bioactive compounds such as alkaloid, phenolic, tannin, etc.

Pineapple peel is farming waste that is rich in bioactive compounds such as flavonoids, alkaloids, tannins, saponin (Mulyani et al., 2021), phenolic, and

terpenoid (Namrata, Sharma, & Sharma, 2017). Currently, pineapple peel has been used for nata de pina (Sutanto, 2012), vinegar (Chalchisa & Dereje, 2021), bioethanol (Tropea et al., 2014), dan eco-enzyme (Ramadani et al., 2019).

Eco-enzyme produced by fermentation of vegetable or fruit waste. Eco-enzymes from fruit peels in Nepal have been tested to have antibacterial activity for *S.aureus* (ATCC 25923), *Bacillus spp*, *Shigella spp*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *E.coli* (ATCC 25922) (Neupane & Khadka, 2019). In addition, the eco-enzyme made from a mixture of orange peel (*Citrus aurantium*) and pineapple peel (*Ananas comosus*) with a ratio of 4:6 was also able to inhibit the growth of *Enterococcus faecalis* bacteria (Mavani et al., 2020). These antibacterial properties make eco-enzyme in Indonesia widely used as a disinfectant liquid, hand sanitizer (Alkadri & Asmara, 2020) and has been applied as a bioactive compound in liquid soap formula made by the home industry (Saifuddin, et al, 2021).

This research will be scientific support that test the antibacterial activity of the phytochemical compound of eco-enzyme in inhibiting and killing the two bacteria that cause acne. The research objectives are made eco-enzyme from pineapple skin, analyze its phytochemical content, and test its antibacterial activity against *S.aureus* and *P.acnes*. The antibacterial test was carried out by dilution to determine the minimum inhibitory concentration (MIC) and diffusion method to examine the effectiveness of inhibition through the diameter of the inhibition zone. Information about the effectiveness of eco-enzyme from pineapple peel in inhibiting the growth of acne-causing bacteria is expected to be useful for further research related to the application of Eco-enzyme in the formulation of anti-acne products.

METHODOLOGY

Materials and Instrumentals

The tools used are laminar airflow (Thermoscientific), autoclave (Tomy SX-700), incubator (Memer+), analytical balance (Mettler toledo), hot plate (Cimarec), vortex (Benchmark), micropipette (Eppendorf), tweezers, Ose needle, spray bottle, bunsen, lighter, oven, ruler and sterile cotton swab (One med).

The materials used in this study included: water, distilled water, pineapple peel, brown sugar, *Staphylococcus aureus*, and *Propionibacterium acnes*, nutrient agar (Difco™), Nutrient Broth (Difco™), Clindamycin 150 mg, Magnesium powder (Merck), Hydrochloric acid (Merck), Ferri chloride (Merck),

Sulfuric acid (Merck), Barium chloride (Merck) and Dragendorff's.

Methods

Eco-enzyme from pineapple peel

An unrotten of pineapple peel used for made of eco-enzyme. Pineapple peel is washed, cut into small pieces, mixed with brown sugar and water (ratio 3:1:10), and stored in a tightly closed plastic container and protected from direct sunlight. The fermentation process was carried out for 3 months. In this process, gas will be produced, so it is sometimes necessary to open the lid of the plastic container to remove the gas. After 3 months, the eco-enzyme was filtered to separate the filtrate from the precipitate which contains pineapple peel. The filtrate is ready to be analyzed for its phytochemical content and antibacterial activity (Ramadani et al., 2019).

Phytochemical Screening

A. Flavonoid Identification

1 ml of eco-enzyme was reacted with 2 mg of magnesium powder (Mg) and 3 drops of 37% HCl. The presence of flavonoid compounds is indicated by the formation of a red, yellow, or orange color.

B. Alkaloid Identification

1 ml of eco-enzyme sample was reacted with 5 drops of Dragendorff's reagent. A positive result is indicated by the formation of orange color in the sample.

C. Tanin Identification

1 ml of eco-enzyme was reacted with 5 drops of 1% FeCl₃ reagent. The presence of tannin compounds is indicated by the formation of blue-black or green-black color.

D. Saponin Identification

10 drops of hot distilled water were put into a test tube containing 1 ml eco-enzyme. Positive results are indicated by the formation of foam which is stable for 30 minutes and does not disappear with the addition of 1 drop of 2N HCl (VH et al., 2021).

Preparation of Mc Farland standard solution

Mc Farland standard solution was prepared by reacting 0.05 ml of 1% BaCl₂ solution with 9.95 ml of 1% H₂SO₄ solution and then homogenized. The turbidity of Mc Farland's solution was used as a standard for the turbidity of the test bacterial suspension (Komansilan, Mintjelungan, & Waworuntu, 2015).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of MIC and MBC on eco-enzyme from pineapple peel was carried out using the dilution method, with the following steps:

1. Prepared 7 test tubes that have been sterilized and labeled I, II, III, IV, V, VI, Ke, Kp, Km, and Ks.
2. The composition of test tubes I to VI is as follows:
3. 1 ml from tube VI solution was poured until 1 ml remained I
4. Each tube was homogenized by vortex, then incubated at 37 °C for 24 hours.
5. 1 ml medium test (Nutrient broth) + 1 ml eco-enzyme was mixed in the eco-enzyme control tube
6. 1 ml medium test (Nutrient broth) + 1 ml sterilized distilled water poured in the solvent control
7. Media control filled 2 test medium
8. The suspense control tube is filled with 2 ml of bacterial suspension
9. Added 1 ml of bacterial suspension in each test tube, except the control tube, then incubated at 37 °C for 24 hours.
10. The determination of MIC is held by observing the turbidity of each solution in the incubated test tube, compared to the control medium solution. The clearest solution is the solution that has the lowest concentration of inhibition of bacterial growth.
11. For MBC analysis, a small amount of solution was taken using a sterile cotton swab and scratched on a petri dish containing NA media and was labeled according to the concentration of the sample. The petri dishes were then incubated at 37 °C for 24 hours. Determination of MBC is based on observations of the number of bacterial colonies growing on NA media. MBC analysis was carried out on test tubes that had been added with bacterial suspension (Fitriana, Fatimah, & Fitri, 2019).

Antibacterial activity test using paper disc diffusion method

Determination of eco-enzyme activity from pineapple peel eco-enzyme as an antibacterial for *S. aureus* and *P. acnes* was carried out using the paper disc diffusion method. First, prepare a bacterial suspension by inoculating bacterial cultures into Nutrient Broth (NB) media and then incubating them for 18-24 hours at 37 °C. After incubation, the bacterial suspension was streaked on the surface of the Nutrient Agar (NA) media on a petri dish using a sterile cotton swab. Disc paper that had been soaked in eco-enzyme solution with a higher concentration of MIC, namely 50%, 75%, 100% v/v, sterile distilled water, and 25% clindamycin solution were placed on NA media. Sterile

distilled water served as a negative control and solvent for the eco-enzyme sample, while the clindamycin 150 mg 25% solution served as a positive control. The inhibition zone was determined after incubation at 37 °C for 24 hours. Antibacterial activity testing was carried out 3 times (triplo) (Syamsurya, *et al.*, 2016, Rosalina, *et al.*, 2018).

Table 1. Composition of the test

Test tube code	Composition of test tube			Concentration of Test tube
	Eco-enzyme (ml)	Sterilized distilled water (ml)	Suspension of bacteria (ml)	
I	1 ml solution of eco-enzyme	1	1	eco-enzyme 50%
II	1 ml solution from tube I	1	1	eco-enzyme 25%
III	1 ml solution from tube II	1	1	eco-enzyme 12,5%
IV	1 ml solution from tube III	1	1	eco-enzyme 6,25%
V	1 ml solution from tube IV	1	1	eco-enzyme 3,125%
VI	1 ml solution from tube V	1	1	eco-enzyme 1,5625%

Data Analysis

The results of the eco-enzyme antibacterial activity test against *S. aureus* and *P. acnes* by diffusion were evaluated using statistical analysis of variance (ANOVA) with Tukey's follow-up test (Minitab 17 Pro software). The statistical test serves as a determinant of the most effective concentration that can inhibit or bactericidal to *S. aureus* and *P. Acnes*.

RESULTS AND DISCUSSION

Eco-enzyme from pineapple peel

The production of eco-enzyme begins with sorting the raw material (pineapple skin), which is not too dry and not rotten. The pineapple skin is then washed to remove impurities, cut into small pieces, put in a plastic container, reacted with brown sugar and water, then tightly closed and stored in a cool place and protected from direct sunlight. This storage process is carried out for 3 months, wherein the first month a lot of gas will be formed from the fermentation reaction so that the lid of the plastic container needs to be opened periodically to release the gas.

The aroma produced is a typical pineapple aroma. According to Rohmah *et al.* (2020), the reaction that occurs in the process of eco-enzyme is fermentation, where the results of this fermentation reaction are

alcohol, acetic acid, and lactic acid. The eco-enzyme obtained in this research can be seen in the Figure 1.



Figure 1. Eco-enzyme from pineapple peel

Phytochemical screening

Phytochemical screening carried out on eco-enzyme samples aims to determine the secondary metabolites contained in the eco-enzyme. The results of phytochemical screening (Table 1) show that the eco-enzyme contains tannins and saponins. Tannins are secondary metabolites of the polyphenol group that can function as antifungals (*Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans*) and antibacterial (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*) (Wafa, Sofiane, & Mouhamed, 2016). The presence of tannin in eco-enzyme can be identified by the reagent solution of iron (III) chloride. The phenol group in tannin will react with Fe^{3+} ions from $FeCl_3$ to form complex compounds that are green-black or blue-black, according to the reaction in Figure 2.

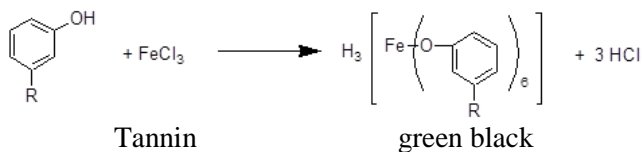


Figure 2. Chemical reaction among tannin and $FeCl_3$

Saponins are complex glycoside compounds that can function as antibacterial, by increasing cell membrane permeability and disrupting the surface tension of bacterial cell walls so that bacteria will experience membrane protein denaturation and eventually lysis (Suerni, Alwi, & Guli, 2013). According to Tagousop et al. (2018), According to Tagousop et al. (2018), saponins are antibacterial (*E.coli*, *S.aureus*, *S.flexneri*), and antifungal compounds (*Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans*).

Determination of MIC and MBC

Determination of MIC on eco-enzyme against *S. aureus* and *P. acnes* was carried out using the liquid dilution method while the determination of MBC was

carried out using the solid dilution method. In liquid dilution, a series of dilutions of eco-enzyme were made with the addition of test medium (distilled water) and test bacteria (*S.aureus* and *P.acnes*), so that a minimum concentration of eco-enzyme was produced which could inhibit the growth of the test bacteria. The minimum concentration of eco-enzyme was seen from the culture results in the test tube which began to appear clear (no bacterial growth). The MIC is then used as a reference for testing with the disc diffusion method. The results of the MIC test can be seen in Figure 3 and Table 2.

Table 2. Phytochemical screening of eco-enzyme

Test	Reagent	Result	Noted
Flavonoid	Mg + HCl 37%	-	Yellow pale
Alkaloid	Dragendorff	-	Yellow pale
Tanin	$FeCl_3$ 1%	+	Green blackish
Saponin	Hot distilled water + HCl 2 N	+	Foam as ± 30 minute



Figure 3. Result of MIC eco-enzyme *P.acnes* (upside) dan *S.aureus* (downside)

In solid dilution, the test bacteria were inoculated on NA media containing eco-enzyme with a certain

concentration variation in order to obtain a minimum concentration that could kill the test bacteria. MBC is characterized by the absence of bacterial colonies growing on solid media. The results of the MBC test (Table 3) showed that all variations in the concentration of eco-enzyme were overgrown with test bacteria, so it can be concluded that eco-enzyme was only able to inhibit the growth of *S.aureus* and *P.acnes* bacteria but was unable to be bactericidal.

Table 3. MIC and MBC test result of eco-enzyme

Test tube	Concentration	MIC test		MBC test	
		<i>S.aureus</i>	<i>P.acnes</i>	<i>S.aureus</i>	<i>P.acnes</i>
I	50%	Clear	Clear		
II	25%	Turbid	Turbid		
III	12,5%	Turbid	Turbid		
IV	6,25%	Turbid	Turbid		
V	3,125%	Turbid	Turbid	Bacteria growth	Bacteria growth
VI	1,5625%	Turbid	Turbid		
Ke	-	Clear	Clear		
Kp	-	Clear	Clear		
Km	-	Clear	Clear		
Ks	-	Turbid	Turbid		

Antibacterial activity test using paper disc diffusion method

The antibacterial test using the diffusion method aims to determine the effectiveness of eco-enzyme in inhibiting the growth of test bacteria. The effectiveness of the inhibition increased along with the larger the inhibition zone (clear zone) produced. Table 3 shows that the effectiveness of eco-enzyme in inhibiting the growth of *S.aureus* and *P.acnes* bacteria is directly proportional to the increase in the concentration of eco-enzyme used. In addition, the inhibition of eco-enzyme against *S.aureus* was greater than that of *P.acnes*. the antibacterial activity of the eco-enzyme produced from this study against *S.aureus* bacteria was lower than that produced by (Neupane & Khadka, 2019), which was 23 mm. The most optimal concentration of eco-enzyme in inhibiting *S.aureus* based on Tukey's further test (95% confidence level) was eco-enzyme 100% (v/v), whereas in *P.acnes* there was no concentration that most significantly inhibited bacterial growth. Several factors that affect the antibacterial activity of compounds in eco-enzymes are the type, origin, and characteristics of the waste used (Neupane & Khadka, 2019; Rasit & Mohammad, 2018) and the length of fermentation time (Mavani et al., 2020).

This result of the antibacterial potential to inhibit *S.aureus* growth has a similar trend with Surtina et al., (2020) which was stated that the greater concentration of the bioactive compound used more inhibit the bacteria. In the eco-enzyme, the most valuable compound to stress the bacteria growth is tannin, one

of the phenolic chemicals. Based on Nurliana & Musta, (2019) the proofed that the inhibition mechanism of these chemicals form an interaction that resulted the protein-phenolic complex, a non-specific chemical linkages. The inhibition strength is related to the concentration of the phenolic content. The low concentration of tannin formed protein-phenols complex with weak bonds so that it immediately decomposed then damaged the cytoplasmic membrane and caused leakage of the cell body. On the other hand, the high concentration, these substances capable to coagulate with cellular proteins and cytoplasmic membrane which finally caused lysis.

Table 3. Eco-enzyme antibacterial activities

Eco-enzyme	Inhibition zone (mm)	
	<i>S.aureus</i>	<i>P.acnes</i>
eco-enzyme 50%	9,67 ± 0,52 ^b	7,00 ± 0,00 ^b
eco-enzyme 75%	10,17 ± 0,41 ^b	8,50 ± 0,55 ^b
eco-enzyme 100%	12,33 ± 1,37 ^a	8,67 ± 0,52 ^b
positive control	6,00 ± 0,00 ^c	28,00 ± 5,33 ^a
Negative control	6,00 ± 0,00 ^c	6,00 ± 0,00 ^b

Note: different letters indicate the most effective concentration in the Tukey test (degree of confident = 95%)

CONCLUSION

Eco-enzyme from pineapple peel produced from this study contains tannins and saponins and has antibacterial activity in the form of the ability to inhibit the growth of acne bacteria *S.aureus* and *P.acnes*. The minimum concentration of inhibition (MIC) of pineapple peel Eco-enzyme is 50%. The most effective concentration to inhibit *S.aureus* was eco-enzyme 100% (v/v) while in *P.acnes* there was no most effective concentration.

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