



# INTERNASIONAL CONFERENCE ON MULTIDISCIPLINARY APPROACHES IN HEALTH SCIENCE

VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)

<https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs>

## The Potential of Organic Materials in the Making of *Eco-Enzyme* as an Inhibitory Power Against *Staphylococcus aureus*

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### Article history

Posted : 2025-09-29

Reviewed : 2025-12-05

Received : 2025-12-13

### ABSTRACT

*Staphylococcus aureus* is a pathogenic bacterium that can cause infection and shows resistance to antibiotics. *Eco-enzyme* is the result of organic waste fermentation that has the potential as a natural antibacterial agent. This study aims to determine the relationship of variations of organic materials in the making of *eco-enzyme* with the inhibitory power of *Staphylococcus aureus*. This type of research is true experimental. Three variations of *eco-enzyme* used are fruit waste (orange peel and pineapple), vegetable waste (mustard greens and cabbage), as well as a mixture of fruit and vegetables, which were fermented for 90 days. The inhibitory test was carried out using the well diffusion method on Nutrient Agar media with pure culture of *Staphylococcus aureus*. The results showed that *eco-enzyme* from fruit waste produced the largest inhibition zone diameter (6.45 mm), followed by vegetable waste (3.88 mm), and mixture (3.21 mm). One-Way ANOVA analysis showed significant differences among the variations, and further LSD test showed that there were significant differences between fruit waste *eco-enzyme* with vegetable waste *eco-enzyme* and mixture *eco-enzyme*. It was concluded that the type of organic material affects the antibacterial ability of *eco-enzyme*, in which fruit waste *eco-enzyme* is the most effective variation. It is recommended to conduct analysis of bioactive compounds, expand tests on other types of bacteria, monitor pH during the fermentation process, as well as develop *eco-enzyme*-based products such as soap or natural antiseptics.

**Keywords:** *eco-enzyme*, organic material, *Staphylococcus aureus*, inhibition zone, fermentation.

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## INTRODUCTION

Bacterial infectious diseases are still a major health problem at the global level, especially with the increasing cases of antibiotic resistance. One of the pathogenic bacteria that is often found in humans is *Staphylococcus aureus*, the cause of various skin infections such as acne, abscesses, scabies, tinea versicolor, up to infections on wounds (Nur Suci Febrianti and Tivani, 2024). A number of studies reported that *Staphylococcus aureus* has shown resistance to several antibiotics, especially the cephalosporin group such as ceftriaxone (100% resistance) and cefoperazone (33.33%). Resistance was also found in aminoglycoside group antibiotics, namely gentamicin, tobramycin, and chloramphenicol, each at 66.66%. Meanwhile, antibiotics from the quinolone and tetracycline groups such as levofloxacin, nitrofurantoin, and tetracycline still showed full sensitivity (Desi, Septa and Hastuti, 2020). In addition to antibiotic resistance, the widespread use of chemical antiseptics can also cause side effects such as irritation and burning sensation on the skin (Varissa *et al.*, 2023). Repeated exposure to chemical antiseptics

is also feared to cause long-term effects on skin health as well as the environment. Therefore, efforts are needed to find new antibacterial sources that are not only effective, but also safe for humans and environmentally friendly.

One potential alternative is the utilization of *eco-enzyme*, a liquid resulting from the fermentation of organic waste such as vegetable and fruit residues with the addition of water and sugar or natural sweeteners (Irianto *et al.*, 2023). This fermentation process produces various active compounds such as alcohol, acetic acid, and enzymes (protease, amylase, lipase) that function to inhibit the growth of pathogenic microorganisms (Galintin, Rasit and Hamzah, 2021). *Eco-enzyme* is generally produced through a simple process that does not require high technology, so it has the potential to be applied in various layers of society, both for household needs and community scale. From an environmental perspective, *eco-enzyme* also offers a dual solution, namely as a natural antibacterial agent as well as a means of organic waste management. Based on data from the National Waste Management Information System (SIPSN)

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in 2023, organic waste dominates the waste composition in Indonesia at 39.64%, with households as the largest contributor (60.53%) (SIPSN, 2023). In Bali, cultural factors also increase the generation of organic waste. Traditional ceremonies that use offerings also produce organic waste in the form of flowers, leaves, and fruits in quite large quantities (Wijaya and Putra, 2021). The abundance of organic waste can be utilized as raw material for making *eco-enzyme*, which at the same time can support efforts to reduce household waste generation.

A number of previous studies have proven the potential of *eco-enzyme* in inhibiting the growth of pathogenic bacteria. *Eco-enzyme* made from pineapple peel has been proven to inhibit the growth of *Staphylococcus aureus* and *Propionibacterium acnes* at a minimum inhibitory concentration (MIC) of 50%, suspected due to the content of tannins and saponins which are antibacterial (Ramadani, Karima and Ningrum, 2022). *Eco-enzyme* made from orange peel also showed antibacterial effectiveness with inhibition zone diameter reaching 22.92 mm against *Staphylococcus aureus* (Poppy

Nurmayanti M *et al.*, 2022). However, most studies are still limited to the use of a single type of organic material, so information regarding the effect of combining several types of organic materials on the antibacterial effectiveness of *eco-enzyme* is still limited. Variations of organic materials can provide synergistic interactions among bioactive compounds, such as flavonoids, tannins, saponins, alkaloids, glucosinolates, up to bromelain, which have the potential to increase antibacterial effectiveness. The combination of several organic materials has the potential to produce *eco-enzyme* with richer bioactive compounds, thereby increasing antibacterial effectiveness compared to *eco-enzyme* made from single material.

Based on the above description, this research was conducted to explore the relationship of variations of organic materials in the making of *eco-enzyme* with the inhibitory power of *Staphylococcus aureus*. The variations used include the combination of orange peel and pineapple peel, the combination of mustard greens and cabbage residues, as well as the combination of all four materials. This study is expected to determine the most

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optimal variation of organic material in producing *eco-enzyme* with antibacterial activity. The results of the study are expected not only to contribute to the development of natural antibacterial alternatives that are safe and environmentally friendly, but also to support organic waste management strategies in Indonesia.

## METHOD

This research was carried out at the Microbiology Laboratory, Environmental Health Department, Health Polytechnic of the Ministry of Health Denpasar, in January – May 2025. This research used a true experimental design with a posttest only control group design. The experimental groups consisted of fruit waste *eco-enzyme* (orange peel and pineapple peel), vegetable waste *eco-enzyme* (mustard greens and cabbage), and mixed *eco-enzyme* (orange peel, pineapple peel, mustard greens, and cabbage) with 100% *eco-enzyme* concentration. Positive control used ciprofloxacin 1%, while negative control used aquadest. The measurement of inhibitory power was carried out by observing the diameter of the inhibition

zone of *Staphylococcus aureus* growth after treatment. The number of treatments was five consisting of three experimental groups and two control groups, with five replications, so that the total number of samples was 25.

The equipment used in this research included a closed container with a capacity of 1,000 ml, stirrer, spoon, petri dish, volume pipette, micropipette, measuring glass, Erlenmeyer flask, test tube, test tube rack, Bunsen burner, inoculating loop, analytical balance, hot plate, autoclave, incubator, refrigerator, and stationery. The materials used in this research included water, sugar, fruit waste (orange peel and pineapple peel), vegetable waste (mustard greens and cabbage), mixture (orange peel, pineapple peel, mustard greens, and cabbage), paper labels, pure culture of *Staphylococcus aureus*, nutrient agar (NA) media, sterile aquadest, ciprofloxacin solution, NaCl 0.85%, sterile cotton swab, aluminum foil, 70% alcohol, cotton, rough paper, suspension of *Staphylococcus aureus*, fruit waste *eco-enzyme* sample, vegetable waste *eco-enzyme* sample, mixed *eco-enzyme* sample.

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This research began with the making of *eco-enzyme* using a plastic container with a capacity of 1,000 ml that had been cleaned and labeled according to the experimental groups. Clean water as much as 600 ml (60% of the container volume) was poured into the container, then added with 60 g of brown sugar (10% of the water weight) and 180 g of organic material pieces (30% of the water weight). The mixture was stirred until homogeneous, then the container was tightly closed and labeled with the production date and harvest date. The fermentation process lasted for 90 days, with opening of the lid and stirring on the 3rd, 7th, and 30th day. After the 90th day, the *eco-enzyme* was ready to be harvested. *Eco-enzyme* liquid samples were taken by stirring the solution evenly, then taking 20 ml using a volume pipette and putting it into an Erlenmeyer flask.

Rejuvenation of *Staphylococcus aureus* bacteria on Nutrient Agar (NA) media. NA media was prepared by weighing 4.2 g of NA powder, dissolving it in 150 ml aquadest, then heating it using a hot plate until homogeneous. The Erlenmeyer flask was covered with cotton

and aluminum foil, then sterilized in an autoclave at 121°C for 15 minutes. The media was poured into sterile petri dishes, left to solidify, then used to grow pure cultures of *Staphylococcus aureus* using the streak plate method. Incubation was carried out at 37°C for 24 hours. Bacterial suspension was made by pouring 5 ml of physiological NaCl 0.85% solution into a test tube. Colonies of *Staphylococcus aureus* from pure culture were taken using a sterile inoculating loop and suspended into the NaCl solution until turbidity was equivalent to the 0.5 McFarland standard. Test media were prepared by pouring 20 ml of NA media into each sterile petri dish, then left to solidify.

The inhibitory test was carried out using the well diffusion method. A sterile cotton swab was dipped into the suspension of *Staphylococcus aureus*, then spread evenly on the surface of NA media until the entire surface was covered with bacteria. After that, five wells were made in each petri dish, then each was dropped with 20 µL of samples with treatment variations: fruit waste *eco-enzyme* 100%, vegetable waste *eco-enzyme* 100%, mixed *eco-enzyme* 100%, ciprofloxacin 1% as positive

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control, and aquadest as negative control. The same treatment was carried out in each replication, then the media was incubated at 37°C for 24 hours.

The test results were observed by looking at the inhibition zone marked by a clear area around the wells. The diameter of the inhibition zone was measured using calipers in millimeters, measured from one side to the other through the center of the well. The value of the inhibition zone diameter was obtained from the reduction between the diameter of the clear area and the diameter of the well. All measurement results were recorded for each treatment and replication as research data. The data on the inhibition zone diameter measurements from the inhibitory test of variations of organic materials in the making of *eco-enzyme* against the growth of *Staphylococcus aureus*, expressed in millimeters (mm), were processed using the tabulating method presented in the form of tables.

Data analysis was carried out with statistical tests with the help of computer software, and the level of significance ( $\alpha$ ) used was 0.05. The stages of data analysis carried out were the Shapiro-Wilk test used

to test the normality of data from the measurement of inhibition zone diameters in each treatment. The test results stated that the data were normally distributed ( $p > 0.05$ ), so Levene's Test of homogeneity was carried out before continuing with parametric tests, the results showed homogeneous data variance ( $p > 0.05$ ), then continued with One-Way ANOVA test to analyze significant differences of the three treatments, and the results obtained showed there was a significant difference ( $p < 0.05$ ), then continued with Post Hoc LSD (Least Significant Difference) test to compare all treatment means that had significant differences.

## RESULTS

The fermentation results of *eco-enzyme* showed differences in color and aroma. Fruit waste *eco-enzyme* was brownish yellow with a strong acidic aroma, vegetable waste *eco-enzyme* was brownish green with a weaker aroma, and mixed *eco-enzyme* was yellowish green with a weak acidic aroma. All replications showed white mold growth on the surface. The results of pH measurements showed that fruit waste *eco-enzyme* had an average

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pH of 3.00, vegetable waste 3.57, and mixture 3.59.

The inhibitory test of *Staphylococcus aureus* showed that the positive control Ciprofloxacin 1% produced an average inhibition zone of 20.76 mm (very strong category), while the negative control Aquadest had no inhibitory power (0 mm). Fruit waste *eco-enzyme* produced an average inhibition of 6.45 mm (medium category), vegetable waste 3.88 mm (weak category), and mixture 3.21 mm (weak category).

Table 1 Diameter of Inhibition Zone of Experimental and Control Groups

	Inhibition Zone Diameter (mm)				Average	Category	Description
	I	II	III	IV			
Fruit Waste <i>Eco-enzyme</i>	7,4	2,9	5,75	7,3	6,45	Medium	Significantly different
Vegetable Waste <i>Eco-enzyme</i>	3,55	3,0	4,85	2,9	3,88	Weak	Significantly different
Mixed <i>Eco-enzyme</i>	4,55	2,1	2,3	3,15	3,21	Weak	Significantly different
Ciprofloxacin 1%	21,2	19,2	20,5	21,95	20,95	Very Strong	Not significantly different
Aquadest	0	0	0	0	0	0	Not significantly different

significant difference among groups ( $p = 0.016$ ). The Post Hoc LSD test showed that fruit waste *eco-enzyme* was significantly different from vegetable waste ( $p = 0.023$ ) and mixture ( $p = 0.007$ ), while vegetable waste and mixture were not significantly different ( $p = 0.511$ ).

Table 2 Results of Data Analysis of Experimental Groups

Data Analysis	Statistic	Sig.	Description
<b>Shapiro-Wilk Normality Test</b>			All three data are normally distributed
Fruit Waste <i>Eco-enzyme</i>			
Vegetable Waste <i>Eco-enzyme</i>			
Mixed <i>Eco-enzyme</i>	0,928	0,586	Data are homogeneous
<b>Levene's Test of Homogeneity of Variance</b>	0,853	0,203	
Based on Mean	0,932	0,610	
Based on Median	1,874	0,196	
Based on Median and with adjusted df	0,672	0,529	Significantly different
<b>One-Way ANOVA Test</b>	0,672	0,547	
Between Groups			
<b>LSD Test</b>			
Fruit Waste <i>Eco-enzyme</i>		0,016	Significantly different
Vegetable Waste <i>Eco-enzyme</i>		0,023	Significantly different
Mixture		0,007	Significantly different
Fruit Waste <i>Eco-enzyme</i> - Vegetable Waste		0,511	Not significantly different
Fruit Waste <i>Eco-enzyme</i> - Mixed			
Vegetable Waste <i>Eco-enzyme</i> - Mixed			

Data analysis showed normal distribution (Shapiro-Wilk  $p > 0.05$ ) and homogeneity (Levene's Test  $p = 0.196$ ).

The One-Way ANOVA test showed a

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## DISCUSSION

*Eco-enzyme* fermented for 90 days has acidic properties with an average pH value ranging from 3.00–3.59. Fruit waste *eco-enzyme* has the highest acidity level (pH 3.00), followed by vegetable waste *eco-enzyme* (pH 3.57) and mixture (pH 3.59). This acidity plays an important role in inhibiting the growth of *Staphylococcus aureus*, because an acidic environment can damage the structure of the cell wall and interfere with bacterial metabolism. This finding is in line with the theory stating that organic acids produced by fermentation, such as acetic acid and citric acid, can reduce pH and create unfavorable conditions for the growth of pathogenic bacteria. In addition to pH, differences in the color and aroma of the fermentation solution were also observed. Fruit waste *eco-enzyme* was brownish yellow with a fresh acidic aroma, vegetable waste *eco-enzyme* was brownish green with a weaker acidic aroma, and the mixture showed a yellowish green color with a relatively weak acidic aroma. This variation shows that the type of organic material used affects the metabolites produced during

fermentation, which ultimately influences antibacterial activity.

The antibacterial test results using the well diffusion method showed that fruit waste *eco-enzyme* produced an average inhibition zone of 6.45 mm (medium category), higher compared to vegetable waste *eco-enzyme* (3.88 mm) and mixture (3.21 mm), both of which were in the weak category. This difference can be explained by the content of bioactive compounds present in each organic material. Orange peel is known to contain limonene, flavonoids, and vitamin C, while pineapple is rich in bromelain, tannins, and saponins. These compounds have strong antibacterial mechanisms, such as damaging cell membranes, disrupting enzyme functions, and reducing bacterial cell wall stability. On the other hand, vegetable waste *eco-enzyme* formulated from mustard greens and cabbage had lower effectiveness even though it contained secondary metabolites such as glucosinolates, flavonoids, and alkaloids. The low antibacterial activity can be related to the relatively small concentration of active compounds and lower acidity level compared to fruit waste

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*eco-enzyme*. Meanwhile, mixed *eco-enzyme* did not show any synergistic effect, even its activity was lower compared to single fruit waste *eco-enzyme*. This indicates the possibility of interactions among compounds that do not mutually support in producing antibacterial effects.

The statistical analysis results support these findings. The Shapiro-Wilk normality test and Levene's Test of homogeneity showed that the data were normally distributed and homogeneous, thus fulfilling the requirements for parametric analysis. The One-Way ANOVA test showed significant differences among treatment groups ( $p = 0.016$ ). The further LSD test showed that fruit waste *eco-enzyme* was significantly different from vegetable waste *eco-enzyme* ( $p = 0.023$ ) and mixture ( $p = 0.007$ ), while vegetable waste *eco-enzyme* and mixture were not significantly different ( $p = 0.511$ ). Thus, it can be concluded that fruit waste *eco-enzyme* has significantly higher antibacterial effectiveness compared to other variations.

The results of this study are consistent with several previous studies. Zaenab, Azizah, and Syamsuddin (2024)

reported that *eco-enzyme* from orange peel and pineapple peel was able to reduce the number of pathogenic bacterial colonies, including *Staphylococcus aureus*. The research by Amiliah, Nurhamidah, and Handayani (2021) also found that Kalamansi orange peel extract can produce an inhibition zone against *Staphylococcus aureus*. Meanwhile, Hidayat, Idawati, and Hardani (2022) reported that *eco-enzyme* from fruit peel can provide significant inhibitory power when applied in solid antibacterial soap formulations. The similarity of these results strengthens the research finding that fruit waste *eco-enzyme* has great potential as a natural antibacterial agent.

## CONCLUSION(S)

*Eco-enzyme* from organic material of fruit waste (orange peel and pineapple peel) showed the highest inhibitory power against *Staphylococcus aureus*, with an average inhibition zone diameter of 6.45 mm, which is included in the medium category. *Eco-enzyme* from organic material of vegetable waste (mustard greens and cabbage) showed lower inhibitory power compared to fruit waste *eco-enzyme*,

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with an average inhibition zone diameter of 3.88 mm, which is included in the weak category. *Eco-enzyme* from organic material of mixed fruit and vegetable waste showed the lowest inhibitory power, with an average inhibition zone diameter of 3.21 mm, which is included in the weak category. The difference indicates that the type of organic material used in the making of *eco-enzyme* affects the antibacterial ability, where fruit waste *eco-enzyme* is the most effective variation in inhibiting the growth of *Staphylococcus aureus* compared to other variations.

## Conflict of Interest

The author(s) declare that they have no conflict of interest.

## Acknowledgment

A thank you to the agencies and parties who supported this research

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