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Phytochemical Analysis and Antibacterial Activity of Eco-Enzyme Produced from Mixed Coffee Pulp and Pineapple Peel Waste

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Abstract: This study aimed to analyze the phytochemical content and antibacterial activity of eco-enzyme produced through the fermentation of a mixed substrate of coffee peels and pineapple peels. This study was a laboratory experiment with repeated observations, including a 2-month eco-enzyme fermentation process, assessment of color and aroma, pH measurement, phytochemical screening, and antibacterial testing against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The results showed that the eco-enzyme was dark brown in color, had an acidic odor, and exhibited a pH ranging from 2.6 to 3.3. The detected phytochemical constituents included alkaloids, flavonoids, and tannins. Antibacterial testing demonstrated that the eco-enzyme derived from the mixture of coffee peels and pineapple peels exhibited activity against *Staphylococcus aureus* and *Escherichia coli*, with all three samples classified as having weak inhibitory activity. The highest inhibition zone diameter against *S. aureus* was observed in sample P1, measuring 5.00 ± 0.26 mm, while the highest inhibition zone against *E. coli* was 5.43 ± 0.78 mm. These values were still substantially lower than those of the positive control (chloramphenicol), which produced inhibition zones of 17.73 ± 2.27 mm and 21.63 ± 0.95 mm, respectively. The bacterial growth inhibition may be attributed to the presence of organic acids in the eco-enzyme, which can reduce the internal cellular pH and disrupt metabolism, as well as to the action of bioactive compounds that may inhibit DNA replication, suppress enzymatic activity, or damage cell membrane permeability. These findings indicate that antibacterial activity was detectable, although still low, highlighting the need to optimize the formulation and fermentation conditions to enhance its antibacterial potential. Nevertheless, this study contributes to the early development of natural non-clinical antibacterial agents through fermentation-based bioconversion.
Keywords: Eco-enzyme, pineapple peel, coffee peel, *Staphylococcus aureus*, *Escherichia coli*

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INTRODUCTION

Waste management in Indonesia remains a serious and unresolved challenge. The accumulation of waste, particularly organic waste, in landfills contributes to global warming, primarily through the emission of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) (Sánchez et al., 2015). This condition may lead to various adverse impacts, including greenhouse gas emissions from anaerobic decomposition processes and environmental pollution caused by toxic leachate (Susilowati et al., 2021). Organic waste generally includes food residues, fruit peels, vegetable waste, and animal-derived waste, which are often not utilized optimally. Household waste, such as organic fruit peel waste, is one of the waste streams that continues to increase annually. The conversion of organic waste into value-added products represents one potential solution for supporting sustainable waste management principles. One promising approach to organic waste management is fermentation to produce value-added bioproducts.

The eco-enzyme fermentation process is influenced by several key parameters

that must be systematically controlled, including temperature, oxygen conditions, gas production, and monitoring of changes throughout fermentation. Fermentation is carried out at room temperature (approximately 25–30°C), which is considered optimal for the activity of fermentative microorganisms in degrading organic materials and producing metabolites such as organic acids (Afecto Gonçalves et al., 2025). The process takes place under semi-anaerobic conditions, in which the container is closed to limit oxygen entry but is not completely airtight, thereby allowing limited gas exchange. This condition supports the growth of facultative anaerobic microorganisms and directs the metabolic pathway toward fermentation (Guillen-Guerrero & de la Rosa-Millan, 2025).

During fermentation, microbial activity produces gases such as CO_2 as metabolic byproducts. Therefore, periodic gas release is required, particularly during the initial phase of fermentation, to prevent pressure buildup inside the container. In addition, physical and chemical changes are monitored throughout the process, including color, aroma, and pH, which serve as indicators of fermentation success and microbial activity. A decrease in pH, along with changes in color and aroma, indicates the formation of fermentation products such as organic acids and secondary metabolites (Guillen-Guerrero & de la Rosa-Millan, 2025).

One of the products generated through this process is eco-enzyme, a liquid derived from the fermentation of organic waste that contains secondary metabolites and natural enzymes (A. Mahdia et al., 2022). Eco-enzyme is produced from organic waste mixed with molasses and water and then fermented for 2–3 months (Neupane & Khadka, 2019). Eco-enzyme solutions generally contain various active substances, including organic acids, secondary metabolites, phenolic compounds, and other bioactive constituents derived from fruit peels or vegetable waste that contribute to microbial activity. These constituents give eco-enzyme potential benefits as an antimicrobial agent. Eco-enzyme produces acetic acid and alcohol during fermentation, and acetic acid (CH_3COOH) is known to inactivate bacteria, viruses, and other pathogenic microorganisms through mechanisms such as inhibition of DNA replication, enzyme (protein) inhibition, and cell membrane degradation (Salsabila et al., 2024). Numerous studies have investigated eco-enzymes produced from single-source fruit organic waste; however, the exploration of other abundant and potentially useful raw materials remains limited and requires further development (Gu et al., 2021).

Among the abundant sources of organic waste that can be utilized as raw materials for eco-enzyme production are coffee peels and pineapple peels. Pineapple peel is an agricultural and household waste rich in bioactive compounds, including flavonoids, alkaloids, tannins, and saponins, which are known to have potential as natural antibacterial agents. Research conducted by Mursidah et al. (2023) demonstrated that pineapple peel extract exhibited strong antibacterial inhibitory activity at concentrations of 10%, 15%, and 20% against *Staphylococcus aureus* and *Propionibacterium acnes* (Mursidah et al., 2023). In addition, Kamila et al. (2024) modified coffee peel-based eco-enzyme by adding papaya peel as a co-substrate. Their results showed that the resulting eco-enzyme contained protease enzymes and bioactive compounds such as tannins and saponins, with a phenol content of 169.8 ppm. This modification also enhanced the antimicrobial activity of the eco-enzyme, as indicated by inhibition zones of 14.43 mm against *Escherichia coli* and 15.33 mm against *Staphylococcus aureus* (Kamila et al., 2024).

Based on previous studies, coffee peel and pineapple peel waste are known to be abundant in Indonesia, particularly in East Java, and to possess individual

antibacterial activity. Therefore, the novelty of this study lies in identifying the most suitable raw material combination for producing eco-enzyme as an antibacterial agent, specifically through the use of mixed coffee peel and pineapple peel substrates. This approach extends previous developments involving coffee peel eco-enzyme (Mulyadi et al., 2023) and coffee peel–papaya peel eco-enzyme (Kamila et al., 2024). Through the evaluation of phytochemical profiles and the antibacterial potential of the eco-enzyme, this study is expected to provide an alternative environmentally friendly and sustainable natural antibacterial material. This research also contributes to the development of agro-industrial waste-based eco-enzymes, provides general insight into eco-enzyme raw materials and phytochemical profiles, and represents an initial investigation into the antibacterial effectiveness of mixed-substrate eco-enzymes. Furthermore, this study supports the achievement of Sustainable Development Goal (SDG) 12.

METHOD

This study was a laboratory-based experimental investigation employing both qualitative and quantitative approaches with repeated measurements. The research was conducted at the Bioprocess Laboratory, Biotechnology Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang. The study aimed to analyze the phytochemical constituents of eco-enzyme produced through the fermentation of mixed coffee peel and pineapple peel waste, and to evaluate its antibacterial activity against selected pathogenic bacteria. The research objects included organic waste materials, namely coffee peel and pineapple peel, which have the potential to produce eco-enzyme through fermentation, as well as pathogenic microorganisms used in the antibacterial assay against *Staphylococcus aureus* and *Escherichia coli*. The sample used in this study was eco-enzyme obtained from the fermentation of mixed organic waste consisting of coffee peel and pineapple peel at a mass ratio of 3:1:10 (organic waste: molasses: water), fermented for 2 months. Eco-enzyme production was carried out in triplicate. These two waste materials were selected as raw materials because they are known to contain bioactive compounds, while the test bacteria were chosen based on their characteristics as representatives of Gram-positive and Gram-negative bacteria commonly associated with infections. All assays conducted in this study were performed in duplicate.

Instruments and Materials

The main instruments used in this study were a UV–Vis spectrophotometer (B-ONE Vis DA), an analytical balance (Ohaus), a laminar airflow cabinet (Hitachi), a pH meter (Lutron, model PH-208), fermentation jars, and various glassware. The principal materials used included Robusta coffee peel waste (*Coffea robusta*), obtained from coffee farmers in Dampit District, Malang; pineapple peel waste obtained from a fruit vendor on Ambarawa Street, Malang; molasses; distilled water; pH 4 and pH 7 buffer solutions; phytochemical reagents such as Dragendorff reagent, NaOH, and $FeCl_3$; bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* obtained from the Microbiology Laboratory of FMIPA UM; Nutrient Agar and Nutrient Broth (Himedia); chloramphenicol paper discs; and blank paper discs.

Eco-Enzyme Preparation

The first stage of the study involved eco-enzyme preparation. Eco-enzyme fermentation followed the formulation described by Neupane and Khadka (2019), by mixing 75 g of dried coffee peel, 75 g of chopped pineapple peel, 50 g of molasses, and 500 mL of water (distilled water). The formulation used a mass ratio of 3:1:10 (fruit

peel waste: molasses: water). All materials were placed into a fermentor consisting of a modified plastic jar fitted with a hose. The hose was connected to a 500 mL bottle containing water, and the fermentor was then tightly sealed. The fermentor was closed using a special lid equipped with a hose connected to a bottle containing 250 mL of water. The container was labeled, and the mixture was fermented for 2 months. After fermentation was completed, the eco-enzyme liquid was filtered to separate the residue. The resulting filtrate, or fermented liquid, was subsequently used for further analyses. In the following stage, the eco-enzyme filtrate was used directly without dilution.

Organoleptic and pH Assessment

Organoleptic testing was conducted using human sensory observation to assess the color and aroma of the eco-enzyme. The pH of the eco-enzyme was measured using a pH meter. The electrode was rinsed with distilled water to ensure that it was free from contaminants. It was then immersed alternately in pH 7 and pH 4 buffer solutions for calibration. After calibration, the electrode was rinsed and dried before being immersed in the sample until a stable pH reading was obtained and the displayed value remained constant.

Qualitative Phytochemical Identification of Eco-Enzyme

Preliminary phytochemical screening of the eco-enzyme included the qualitative identification of alkaloids, flavonoids, tannins, and saponins. For alkaloid identification, 1 mL of eco-enzyme was placed into a test tube, followed by the addition of 5 drops of Dragendorff reagent. The presence of alkaloids was indicated by the formation of an orange to reddish-brown precipitate. For flavonoid identification, 1 mL of eco-enzyme was added to a test tube, followed by 7 drops of 2% NaOH. The presence of flavonoids was indicated by the appearance of a yellow, orange, or red color. For tannin identification, 1 mL of eco-enzyme was placed into a test tube and mixed with 5 drops of 1% $FeCl_3$. A sample was considered positive for tannins if the color changed to blue-black or greenish-black. For saponin identification, 10 drops of hot distilled water were added to a test tube containing 1 mL of eco-enzyme. The presence of saponins was indicated by the formation of stable foam lasting for 10 minutes.

Antibacterial Assay

The agar disc diffusion method was used to evaluate the antibacterial activity of the eco-enzyme against *E. coli* and *S. aureus*, with chloramphenicol as the positive control. For bacterial suspension preparation, one loopful of bacterial culture was aseptically inoculated into Nutrient Broth and incubated at 37°C until an OD600 of 0.6 was achieved, as measured using a UV–Vis spectrophotometer, corresponding to approximately 3 McFarland standard units. For antibacterial testing, 100 µL of bacterial suspension was spread evenly onto Nutrient Agar using sterile cotton buds. Blank paper discs loaded with 20 µL of eco-enzyme filtrate and chloramphenicol discs as the positive control were then placed on the agar surface. DMSO-loaded paper discs were used as the negative control. The inhibition zones were measured using a caliper after incubation at 37°C for 24 h.

Data Analysis

Phytochemical test data were analyzed descriptively by indicating the presence or absence of secondary metabolites, such as alkaloids, flavonoids, saponins, and tannins, using (+/–) notation. Meanwhile, antibacterial assay data were analyzed quantitatively by measuring the diameter of the inhibition zones (mm), which were

presented as mean ± standard deviation from repeated measurements of the eco-enzyme samples.

RESULTS AND DISCUSSION

Characteristics of the Eco-Enzyme Produced from Coffee Peel and Pineapple Peel

Fermentation of a mixed waste substrate consisting of coffee peel and pineapple peel for 2 months produced highly similar characteristics across all three replicates. The resulting eco-enzyme was dark brown to brownish-black in color and had an acidic odor, as shown in Table 1 and Figure 1. The acidic odor of the eco-enzyme is closely related to its pH value, which reflects the acidity or alkalinity of the solution. pH is inversely related to the concentration of organic acids; therefore, a lower pH indicates a higher organic acid content in the eco-enzyme. A low pH may thus serve as an indicator of elevated concentrations of organic acids such as acetic acid and citric acid (A. Mahdia et al., 2022). The eco-enzyme produced in this study can be categorized as a well-fermented product because it met the criteria of a good-quality eco-enzyme, namely a cloudy brown color, a fruity acidic aroma, and acidic properties with a pH value of < 4 (Mahdia et al., 2022).

Table 1. Characteristics and pH values of eco-enzyme produced from coffee peel and pineapple peel

Sample Replicate	Color	Aroma	pH
Replicate 1 (P1)	Dark brown	Acidic pineapple vinegar-like odor	3.37
Replicate 2 (P2)	Dark brown	Acidic pineapple vinegar-like odor	2.71
Replicate 3 (P3)	Brown	Acidic pineapple vinegar-like odor	2.61

Figure 1. Fermentation products of the eco-enzyme from coffee peel and pineapple peel in replicates 1, 2, and 3.

Phytochemical Content of the Eco-Enzyme Produced from Coffee Peel and Pineapple Peel

The results of the phytochemical screening of the eco-enzyme are presented in Table 2.

Table 2. Identification of phytochemical compounds in the eco-enzyme produced from coffee peel and pineapple peel

Phytochemical Content	Reagent	Identification Result	Sampel		
			P1	P2	P3
Alkaloids	Dragendorff	Formation of an orange precipitate (Habibi, Firmansyah, and Setyawati 2018)	+	+	+

Phytochemical Content	Reagent	Identification Result	Sampel		
			P1	P2	P3
Flavonoids	2% NaOH	Formation of an orange to reddish-brown color (Kusnadi and Devi 2017)	+	+	+
Tannins	1% FeCl3	Formation of a greenish-black color (Halimu, S.Sulistijowati, and Mile 2020)	+	+	+
Saponins	Distilled water	Formation of stable foam for 10 minutes (Shaikh and Patil 2020)	-	-	-

Note: (+) indicates a positive result; (-) indicates a negative result.

Phytochemical testing was conducted to identify the compounds present in the eco-enzyme produced from the mixture of coffee peel and pineapple peel. As shown in Table 2, all three replicate samples (P1, P2, and P3) tested positive for alkaloids, flavonoids, and tannins. The presence of these compounds is consistent with the natural characteristics of the raw materials, as coffee peel and pineapple peel are known to contain various secondary metabolites, including alkaloids, tannins, flavonoids, and steroids (Juariah et al., 2018).

These phytochemical compounds exhibit different antibacterial mechanisms. Alkaloids inhibit bacterial growth by intercalating into DNA, thereby interfering with DNA replication and acting as topoisomerase inhibitors (Cushnie et al., 2014). Flavonoids act by disrupting cell membrane integrity and forming complexes with proteins, which can interfere with metabolic processes (Cushnie & Lamb, 2005). Tannins, meanwhile, alter membrane permeability and inhibit enzymatic activity through protein precipitation (Scalbert, 1991). However, the concentration of phytochemical compounds also plays an important role in determining antibacterial effectiveness. In the present study, only qualitative testing was performed, without quantifying the concentration of each phytochemical compound. Therefore, although qualitative analysis confirmed the presence of these compounds, their antibacterial activity may still be low.

In contrast, saponin testing in all three eco-enzyme replicates yielded negative results, as indicated by the formation of only a small amount of foam that persisted for approximately one minute after shaking. A positive indication of saponins is defined by the presence of stable foam lasting at least 10 minutes (Shaikh & Patil, 2020). The absence of detectable saponins may be attributed to their low abundance in the raw materials used, such as coffee peel and pineapple peel, such that their concentration decreased further during fermentation and was no longer visually detectable.

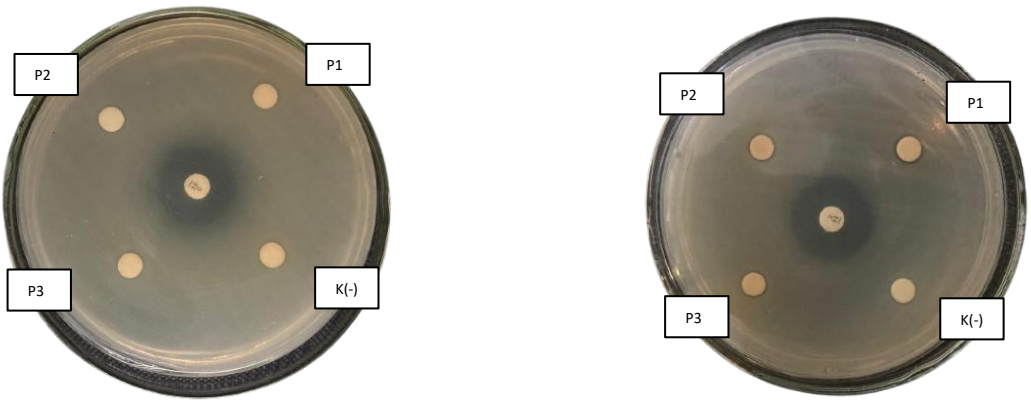
Antibacterial Activity of the Eco-Enzyme Produced from Coffee Peel and Pineapple Peel

The antibacterial activity test was conducted to evaluate the inhibitory effect of the eco-enzyme on the test bacteria. The inhibition assay against *Staphylococcus aureus* and *Escherichia coli* was performed using the disc diffusion method. This method offers several advantages: it is practical and easy to perform, has good sensitivity for aerobic and facultative anaerobic bacteria, allows straightforward interpretation of results, and does not require specialized equipment. Therefore, it is well suited for studies investigating the inhibitory activity of antibacterial agents against bacterial growth (Firman et al., 2023).

Table 5. Antibacterial activity of the eco-enzyme against *Staphylococcus aureus* and *Escherichia coli*

Bacteria	Sample	Inhibition Zone Diameter (mm)
Staphylococcus aureus	K (+)	17.73 ± 2.27
	P1	5.00 ± 0.26
	P2	4.87 ± 0.15
	P3	4.97 ± 0.47
Escherichia coli	K (+)	21.63 ± 0.95
	P1	5.43 ± 0.78
	P2	5.13 ± 0.59
	P3	5.13 ± 0.59

Antibacterial activity was evaluated against two bacterial species, namely *Staphylococcus aureus* (SA) as a Gram-positive bacterium and *Escherichia coli* (EC) as a Gram-negative bacterium. The results are presented as inhibition zone diameters formed around the test discs, indicating the effectiveness of the eco-enzyme samples in suppressing bacterial growth. Based on the data in Table 5, the positive control (K+) produced substantially larger inhibition zones, namely 17.73 ± 2.27 mm for *S. aureus* and 21.63 ± 0.95 mm for *E. coli*. These findings indicate that the testing method functioned properly and that the standard antibacterial agent used as the positive control had high effectiveness.



(a) (b)

Figure 2. Antibacterial activity of the eco-enzyme against (a) *Staphylococcus aureus* and (b) *Escherichia coli*; negative control: DMSO

Among the eco-enzyme samples, P1 exhibited the largest inhibition zone diameter against both test bacteria compared with P2 and P3. Against *S. aureus*, eco-enzyme P1 produced an inhibition zone of 5.00 ± 0.26 mm, whereas P2 and P3 produced zones of 4.87 ± 0.15 mm and 4.97 ± 0.47 mm, respectively. Against *E. coli*, P1 also showed the highest inhibition zone diameter at 5.43 ± 0.78 mm, followed by P2 and P3, both of which showed identical inhibition zone diameters of 5.13 ± 0.59 mm.

Based on these results, all eco-enzyme samples demonstrated weak antibacterial activity, as indicated by inhibition zones of < 10 mm (Davis & Stout, 1971). As an initial screening of the antibacterial activity of the coffee peel–pineapple peel eco-enzyme, these findings suggest low intrinsic antibacterial potential. However, this may also be associated with non-optimal concentration (Balouiri et al., 2015), poor diffusion in the agar medium, or bacterial resistance (Andrews, 2001). In addition,

many bioactive compounds exhibit strain-specific activity and may require optimization or synergistic combinations to produce significant antibacterial effects (Wagner & Ulrich-Merzenich, 2009).

The antibacterial activity of the eco-enzyme is strongly influenced by the synergistic action of the compounds it contains, including organic acids that are responsible for lowering the pH of the eco-enzyme. The presence of these acids contributes to the acidic nature of the eco-enzyme, which in turn affects enzymatic activity and the degradation capacity toward target compounds. Although acidic pH may enhance antibacterial activity by inhibiting bacterial cell metabolism through the dissociation of acidic compounds absorbed by cells, thereby lowering intracellular pH, excessively low pH may also reduce antibacterial activity itself (Kundukad et al., 2017). This is because, at very low pH, the majority of acidic compounds become dissociated in the surrounding environment, reducing their ability to penetrate the cell membrane. In addition, phytochemical compounds such as flavonoids and phenolics may degrade under highly acidic conditions, which may lead to reduced antibacterial activity (Friedman, 2007). Therefore, optimization of the eco-enzyme production process and quantification of phytochemical compounds are needed to achieve optimal antibacterial activity. Nevertheless, this study provides preliminary empirical evidence regarding the phytochemical characteristics and antibacterial activity of an eco-enzyme based on a mixture of coffee peel and pineapple peel.

CONCLUSION

This study demonstrated that an eco-enzyme produced from a mixture of coffee peel and pineapple peel was successfully prepared, as indicated by its characteristic eco-enzyme properties, namely brown color, acidic aroma, and pH < 4, as well as the presence of several phytochemical compounds, including alkaloids, flavonoids, and tannins. The antibacterial activity of this eco-enzyme against *Staphylococcus aureus* and *Escherichia coli* was low, indicating that optimization of the eco-enzyme production process is necessary to improve its antibacterial performance. Nonetheless, this study provides an initial assessment of the antibacterial effectiveness of eco-enzyme produced from mixed organic waste materials.

RECOMMENDATION

For future studies, it is recommended that the preparation of eco-enzyme samples in replicates 1, 2, and 3 be preceded by homogenization in a single container so that, when divided into three replicate samples, each replicate exhibits more uniform eco-enzyme characteristics. In addition, quantitative analysis of each phytochemical compound is needed to evaluate its correlation with the low antibacterial activity observed.

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