Effectiveness of the Ecoenzym Produced from Pineapple and Orange Peel Waste as a Floor Cleaning Agent

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ABSTRACT

Ecoenzyme is one of the products used to process organic waste that we often encounter in our daily lives. This study investigates the effectiveness of Ecoenzyme made from pineapple and orange peel waste as an antibacterial agent, with the aim of finding the optimal results that can later serve as a reference for the development of floor cleaning products. Ecoenzyme is produced by fermentation of organic materials using molasses, pineapple and orange peel waste to water ratio of 1:3:10 over a period of three months. Antibacterial tests against Staphylococcus aureus and Escherichia coli were performed using the disk diffusion method with different concentrations of ecoenzyme (10%, 25%, 50%, 75% and 100%). The results showed that ecoenzyme concentrations of 75% and 100% produced inhibition zones greater than 20 mm, which are classified as very strong against both bacteria. The phenolic, tannin and saponin contents in ecoenzyme play a significant role in its antibacterial activity. ANOVA statistical analysis confirmed the significant effect of ecoenzyme concentration on inhibition zone diameter. This study shows that the most effective concentrations of ecoenzyme for antibacterial activity are 75% and 100%, with inhibition zones larger than 20 mm (very strong). Therefore, ecoenzyme from pineapple and orange peel waste has great potential to be developed into a commercial floor cleaning product. Its high antibacterial efficacy, especially at 75% and 100% concentrations, provides an advantage as an environmentally friendly natural ingredient.

Keywords: Ecoenzyme, Fruit peel waste, Floor cleaner

INTRODUCTION

Ecoenzymes are defined as complex organic compounds consisting of protein chains (enzymes), organic acids, and mineral salts [1]. These compounds play a role in the process of preparation, decomposition, transformation, and catalysis of various chemical reactions [2]. Ecoenzymes can be produced from organic waste such as fruit and vegetable peels, for example orange or pineapple peels. Utilising these readily available organic waste materials for ecoenzyme production can contribute to reducing environmental waste. Orange peels, for instance, are abundant in vitamin C and also contain significant levels of essential oils and pectin [3]. Similarly, pineapple is a rich source of vitamin C and contains the enzyme bromelain, which is found in all parts of the pineapple plant [4].

The production of multi-hydrolytic enzymes, including amylase, protease, and lipase, from ecoenzymes derived from orange and pineapple peels has been shown to facilitate the decomposition of wastewater [5]. Extracellular hydrolytic enzymes exhibit high stability, resistance to chemicals, and the capacity to function across a broad temperature range, a property that enables their survival outside the confines of the cell wall [1]. As stated in [6], ecoenzymes have been successfully produced from various types of fruit peel waste, with the highest quality results obtained from pineapple and orange peels. The utilisation of ecoenzymes in detergent remediation has been demonstrated, underscoring their potential application in environmental engineering.

As posited by [7], ecoenzymes are defined as fermentation products in the form of solutions that are derived from organic waste. These ecoenzymes find application in a variety of contexts, including as floor cleaning fluids, vegetable and fruit cleaners, insect repellents, and plant fertilisers.The antibacterial properties of ecoenzymes are attributed to the presence of acetic acid (H3COOH) within the molecule, which functions as an antibacterial agent [8]. The categorisation of a substance as antibacterial is contingent upon its capacity to impede or halt bacterial growth, or to induce bacterial death. The determination of antibacterial activity is typically facilitated by the implementation of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) methodologies [9].

The present study aims to assess the effectiveness of ecoenzymes as antibacterials. To this end, the study will measure the ability of ecoenzymes at various concentrations and exposure times to inhibit the growth zone of pathogenic bacteria.The study's results will be used as a reference for the development of ecoenzym products made from pineapple and orange peels commercially as a floor cleaner.

MATERIALS AND METHODS

Preparation of Tools and Materials

The instruments utilised in this study encompassed test tubes, petri dishes, Pasteur needles, micropipettes, blue tips, yellow tips, and sterile tweezers. The materials required for the study comprised Nutrient Agar (NA) media, Nutrient Broth (NB), Muller Hinton Agar (MHA), distilled water, and blank discs. The NA media was prepared by dissolving 28 g of NA powder in 1 L of distilled water and heating to boiling. The NB medium was prepared by dissolving 39 g of NB powder in 1 L of distilled water and homogenising the mixture. The MHA medium was prepared by dissolving 38 g of MHA powder in 1 L of distilled water and heating to boiling. All three media, tips, and tweezers were sterilised by autoclaving at 121°C for 15 minutes at 1 atm pressure. The test bacteria used were Staphylococcus aureus and Escherichia coli from the culture stock of BIOME Microbiology Laboratory, Universitas Airlangga.

Preparation of Ecoenzymes

The ecoenzymes are manufactured from orange and pineapple peel waste, as evidenced by research on various fruit peel waste mixtures (Gasperz and Fitrihidajati, 2022). The ecoenzymes are composed of the following ratio of ingredients: 1 (molasses/sugar) : 3 (waste material) : 10 (water/aquades) or 1: 3 : 10. The materials utilised to produce 1 litre of ecoenzym included 150 grams of pineapple peel, 150 grams of orange peel, 100 grams of molasses, and 1 litre of distilled water.

The procedure entailed the preparation of the necessary tools and materials, followed by the initial filling of the jar with 1 litre of distilled water. The pineapple and orange peels were meticulously cut into small pieces, with each piece being weighed to ensure an exact measurement of 150 grams. The molasses was weighed and added to the jar containing the distilled water. The pineapple and orange peels were then added to the jar and thoroughly stirred to ensure uniform distribution. The container is then filled to a capacity of 2/3, allowing space for the fermentation process. Following this, the pH meter and thermometer are prepared for the initial pH and temperature measurements of the ecoenzymes. Once these measurements are completed, the ecoenzymes are sealed and left to ferment for a period of three months.

During the fermentation process, the temperature gradually decreased from 30°C in the first month to 29°C in the second month, and further declined to 28°C in the third month.

Harvesting of Ecoenzym

Following a three-month fermentation process, during which the conditions are meticulously maintained, the next stage is harvesting. Harvesting is achieved by filtering the fermented liquid from the fruit peel pulp to obtain the liquid ecoenzymes that are ready for use. The purpose of this filtration process is to separate the enzyme-rich fermentation liquid from the superfluous pulp, thereby ensuring a purer and more effective ecoenzyme.

Preparation of Ecoenzym Concentration Series

In this study, the term 'concentration' refers to the quantity of ecoenzymes utilised as a floor cleaning concentrate. The study was conducted with five distinct concentrations, each of which was repeated on three occasions. The control group comprised 70% phenol. Ecoenzymes were dissolved in water (1 L volume), with the following concentrations:

- A: 0% = Control (70% phenol)
- B: 10% = 100 ml ecoenzym/L
- C: 25% = 250 ml ecoenzym/L
- D: 50% = 500 ml ecoenzym/L
- E: 75% = 750 ml ecoenzym/L
- F: 100% = pure ecoenzymes without water solution.

Inoculation of Test Bacterial Isolates

At this stage, the rejuvenation of S. aureus and E. coli test bacterial isolates was initiated by growing each isolate on NA media in a test tube and incubating it for 24 hours at 37°C. After incubation, both isolates were inoculated into sterile NB media and incubated again for 24 hours at 120 rpm at 37°C. The bacterial density was then measured using a spectrophotometer at a wavelength of 600 nm with an OD value of 0.1.

Antibacterial Activity Test

Antibacterial activity testing was carried out by the disc diffusion method using a blank disc. Bacterial isolates of S. aureus and E. coli were inoculated onto solid MHA media in Petri dishes by swab method. Ecoenzymes with various concentrations, which had been prepared in advance, were poured into a blank disc and placed on the media. Each petri dish contained one type of test bacterial isolate and three variations of ecoenzyme concentration, with three repetitions in different petri dishes. The petri dishes were then subjected to an incubation process at a temperature of 30°C for a duration of 24 hours, with the objective of enabling the bacteria to grow evenly and form inhibition zones. The zone of inhibition was then measured with calipers, with measurements being taken on both the horizontal and vertical sides.

Data Analysis

The data obtained in this study is the inhibition zone formed around the paper disc due to the antibacterial activity of ecoenzymes against pathogenic bacteria S. aureus and E. coli. The data will be statistically analysed using SPSS for the inhibition zone value or number, and the inhibition zone image will be descriptively analysed.

RESULTS

Ecoenzyme Manufacturing Result

The manufacturing process of ecoenzymes necessitates the utilisation of the raw materials enumerated in the method chapter and illustrated in Fig 1, part (a). Subsequent to a three-month ecoenzym manufacturing process (fermentation), ecoenzym harvesting was undertaken. The ecoenzym fermentation process was conducted within a gallon bottle, which was filled to a capacity of ¾. The transformation in colour during the ecoenzym fermentation process is demonstrated in Figure 1, parts (b) and (c).



(a) Ecoenzyme Raw Materials (b) Preparation of Ecoenzymes (c) Harvested Ecoenzymes

Figure 1. Result of Ecoenzym Production

Results of Analysis of Ecoenzym Active Ingredient Content

This research focuses on making ecoenzymes as floor cleaners with antibacterial properties. Therefore, the active ingredient content of ecoenzymes made from pineapple and orange peels was examined. The test results are presented in Table 1.

Table 1. Test Results of Active Ingredients of Pineapple and Orange Peel Ecoenzymes

Parameter	Repeat			Average
	1	2	3	
Phenol (mg GAE/ml)	71,28	70,00	72,56	71,3
Tannins (mg GAE/ml)	33,12	32,12	34,12	33,1
Saponin (mg/L)	15,69	16,69	14,00	15,5

The Results of The Ecoenzym Antibacterial Activity Test

In the ecoenzym effectiveness test against S. aureus and E. coli bacteria, five distinct concentrations were utilised, with three repetitions at each concentration, in addition to 70% phenol as a control. The treatment was conducted with an incubation time of 1x24 hours. Figure 2 below shows the comparison between ecoenzymes and water used in the observation.



Figure 2. Ecoenzym Concentration Series

The results of the ecoenzym bacteria test with various concentrations are shown in Table 2 below.

Table 2. Antibacterial Activity Test Results of Pineapple and Orange Peel Ecoenzymes

Test bacteria	Concentration	Average pH	Inhibition Zone Diameter			Average Inhibition Zone Diameter
			1	2	3	
S. aureus	Control		27,9	27,6	28,1	27,9
	10%	3,24	0,0	0,0	0,0	0,0
	25%	3,14	0,0	0,0	0,0	0,0
	50%	3,11	10,4	10,2	10,6	10,4
	75%	3,10	22,8	22,6	23,0	22,8
	100%	3,14	27,6	27,8	27,4	27,6
E. coli	Control		26,6	26,4	26,8	26.6
	10%	3,24	0,0	0,0	0,0	0,0
	25%	3,14	0,0	0,0	0,0	0,0
	50%	3,11	23,9	24,1	23,7	23,9
	75%	3,10	24,3	24,1	24,5	24,3
	100%	3,14	26,3	26,5	26,1	26,3

The results of the analysis of variance (ANOVA) statistical testing demonstrated that the concentration of ecoenzymes exerted a significant effect on antibacterial activity against S. aureus and E. coli, as evidenced by the observed discrepancy in the diameter of the inhibition zone formed between concentrations. This can be seen in Figure 3 and Figure 4 below.

ANOVA

ANOVA – Diameter_Zona_Hambat

Cases	Sum of Squares	df	Mean Square	F	p
Konsentrasi	2568.044	5	513.609	16809.018	< .001
Residuals	0.367	12	0.031		

Note. Type III Sum of Squares

Figure 3. Anova test results of the significance of ecoenzymes on antibacterial activity against S. aureus bacteria.

ANOVA

ANOVA – Diameter_Zona_Hambat

Cases	Sum of Squares	df	Mean Square	F	p
Konsentrasi	2572.245	5	514.449	19291.837	< .001
Residuals	0.320	12	0.027		

Note. Type III Sum of Squares

Figure 4. Anova test results of the significance of ecoenzymes on antibacterial activity against E. coli bacteria.

DISCUSSION

The study employed a positive control of phenol, with a concentration of 70%, to facilitate a comparison of the antibacterial activity of S. aureus and E. coli with that of pineapple and orfange peel ecoenzymes. The diameter of the inhibition zone for the phenol positive control was measured, resulting in an average of more than 20 mm on both bacteria. This finding indicates that the inhibition of phenol is classified as very strong. Phenol is frequently employed as a standard for evaluating the effectiveness of disinfectants due to its capacity to eliminate test microorganisms at low concentrations (2-5%). Phenol possesses bactericidal, tuberculoisidal, fungicidal, and virucidal properties against lipophilic viruses [10].

The results of the manufacturing process made from orange peel and pineapple peel waste that refers to the recipe with a ratio of 1: 3: 10, namely 1 (molasses): 3 (fruit peel waste material): 10 (water). The manufacture of ecoenzymes in this study has a volume of 2 litres. After 3 months of ecoenzym making process (fermentation), ecoenzym harvest was conducted. The harvest showed an average pH of 3.14 at 100% ecoenzym concentration. The ecoenzym fermentation process was carried out in a gallon bottle filled as much as ¾ part.

The antibacterial activity test of pineapple and orange peel ecoenzymes with five different concentrations revealed varying degrees of activity, as evidenced by the inhibition zone formed. At concentrations of 10% and 25%, no inhibition zone was observed, indicating that these ecoenzymes were ineffective against S. aureus and E. coli at these concentrations. However, at concentrations of 50% and 75%, the ecoenzymes demonstrated significant antibacterial activity, producing inhibition zones of 10.4 mm and 22.8 mm against S. aureus, respectively, and 23.9 mm and 24.3 mm against E. coli. Furthermore, at 100% concentration, ecoenzym produced an inhibition zone of 27.6 mm against S. aureus and 26.3 mm against E. coli, indicating a high level of antibacterial activity.

When compared with a commercial floor cleaner containing 0.1% benzalkonium chloride as the primary active ingredient, which demonstrated inhibition zones of 15–20 mm against *Staphylococcus aureus* [11]. The antibacterial effectiveness of orange and pineapple peel ecoenzymes at 100% concentration showed comparable activity. According to Davis and Stout, inhibition zones with diameters of >14 mm are classified as strong, 10–14 mm as moderate, 5–9 mm as weak, and <5 mm as inactive [11]. The observed differences in antibacterial activity may be attributed to the distinct nature of the active compounds involved. The commercial floor cleaner contains synthetic benzalkonium chloride at a controlled concentration, whereas the antibacterial activity of ecoenzymes depends on naturally occurring compounds such as limonene and bromelain, whose concentrations may vary depending on the raw materials used. Benzalkonium chloride exhibits strong bacteriolytic activity even at low concentrations due to its potent membrane-disrupting properties. In contrast, the antibacterial effect of ecoenzymes is primarily associated with their acidic pH and the presence of bioactive compounds produced during the fermentation process.

Research [12] has demonstrated the efficacy of pineapple peel-based ecoenzymes in combating various bacterial strains, including gram-positive bacteria such as Staphylococcus epidermidis, S. aureus, and Propionibacterium acnes, as well as gram-negative bacteria such as Pseudomonas aeruginosa and Pantoea stewartii. The study also highlights the effectiveness of ecoenzymes against fungal species, including Lasiodiplodia theobromae, Trichophyton rubrum, and Colletotrichum capsici. Conversely, ecoenzymes derived from orange peel have been observed to demonstrate antibacterial activity against gram-positive bacteria S. aureus and Streptococcus pyogenes, gram-negative bacteria E. coli, Salmonella typhi, P. aeruginosa, and P. stewartii, and fungi Aspergillus niger, Fusarium spp., Cladosporium spp., and Colletotrichum capsici [13].

Staphylococcus aureus and Escherichia coli are frequently utilised as model organisms for antimicrobial testing due to their prevalence as top five pathogenic microorganisms.Staphylococcus aureus is a gram-positive bacterium that possesses a cell wall structure comprising of cytoplasm, peptidoglycan containing lipids and polysaccharides, and an outer layer of peptidoglycan coated with teichoic acid [14]. In contrast, E. coli bacteria are classified as gram-negative bacteria, characterised by a cell wall structure consisting of three

layers: the inner lipoprotein layer, the lipopolysaccharide and phospholipid layers, and the outer layer of thin peptidoglycan [15].

The findings of the study indicated that ecoenzymes exhibited a robust acidic pH profile, with an average value ranging from 3.10 to 3.24. The active principles within ecoenzymes remain under laboratory analysis, with further deliberations to be conducted upon the availability of the analytical outcomes.Ecoenzyme concentrations ranging from 10% to 100% exhibited marginal variations in the results, remaining within the strong acid pH category.

In addition to pH, temperature also plays an important role in determining the antibacterial activity of pineapple and orange peel ecoenzymes. Temperature is an important factor affecting the antibacterial activity of ecoenzymes derived from mixed pineapple and orange peels. According to [16], the antibacterial activity of ecoenzymes is optimal at temperatures ranging from 25–35°C and pH values between 3.0 and 3.5. At temperatures above 40°C, bromelain and protease enzymes undergo denaturation, resulting in a significant reduction in the inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. This occurs because enzymes are thermolabile proteins; therefore, exposure to high temperatures alters their tertiary structure and causes the loss of catalytic function.

These findings are consistent with the study by Suprayogi [17], which stated that ecoenzyme fermentation should ideally be carried out at room temperatures of 27–30°C. Temperatures outside this range may slow the fermentation process and affect the production of organic acids, resulting in a final pH that is less effective in inhibiting the growth of pathogenic bacteria. Therefore, ecoenzymes should be stored at cool room temperatures and protected from direct heat exposure to maintain enzyme stability and antibacterial effectiveness.

ANOVA test results for S. aureus inhibition zone diameter showed an F value of 16809.018, indicating significant differences between concentrations.The p value <0.001 indicates that the difference between concentrations in the diameter of the inhibition zone is significant, thereby allowing the rejection of the null hypothesis. Conversely, the ANOVA test results for the diameter of the inhibition zone of E. coli showed an F value of 19291.837 and a p value <0.001, indicating a significant effect of ecoenzyme concentration on antibacterial activity. The difference in inhibition zone diameter between concentrations was statistically significant at the 0.05 level. This finding suggests that an increase in ecozyme concentration results in a corresponding increase in the diameter of the inhibition zone, indicating a direct relationship between ecozyme concentration and antibacterial activity.

Ecoenzymes effectively inhibit the growth of various microorganisms [18]. In addition, ecoenzymes are also effective in reducing various waste treatment parameters such as BOD, COD, TDS, nitrate, nitrite and ammonium in waste [19]. The antimicrobial activity in ecoenzymes shows its potential as a versatile cleaner and environmentally friendly disinfectant, as it is produced from organic waste and leaves no harmful residue due to the presence of its antibacterial activity [20]. Each type of organic waste, both fruit and vegetables, has contents that can influence the antibacterial activity of ecoenzymes. Based on the analysis of the active ingredients of ecoenzymes from pineapple and orange peels, the content of phenol was 71.3 mg GAE/ml, tannin was 33.1 mg GAE/ml and saponin was 15.5 mg/L. The three types of compounds found in ecoenzymes are classified as antibacterial agents.

Variations in the raw materials used for ecoenzyme production have a significant influence on antimicrobial activity. Ecoenzymes produced from a combination of pineapple and orange peels demonstrated the highest inhibitory activity, particularly at a 100% concentration. This effect is attributed to the synergistic interaction between bromelain enzymes derived from pineapple peel and limonene compounds present in orange peel, which contribute to pH reduction and disruption of bacterial cell walls. In contrast, ecoenzymes produced from other raw materials, such as banana and watermelon peels, exhibited higher final pH values and weaker antibacterial activity. Additionally, ecoenzymes derived from papaya peel showed moderate antibacterial activity due to the presence of papain enzymes. Therefore, the composition of raw materials containing high levels of organic acids and proteolytic enzymes is considered a major factor contributing to the antibacterial effectiveness of ecoenzymes [21].

The process of inhibiting bacterial growth begins with the destruction of its cell wall.Phenol, for example, has been shown to inhibit bacterial growth by breaking peptidoglycan bonds, damaging cell walls, and disrupting permeability and protein function, which causes damage to the bacterial cell wall [22].Secondary metabolite compounds, such as saponins, can penetrate the bacterial cell membrane after the cell wall is damaged, disrupt the cytoplasmic membrane, and inhibit bacterial growth [23]. Subsequent entry of the tannin compound into the bacterial cell results in the targeting of its core, leading to the lysis of bacteria with compromised cell membranes.Tannin compounds have the capacity to coagulate, denature, and precipitate proteins, in addition to inactivating enzymes and damaging bacterial genetic material, thereby rendering it inactive. Tannins have also been shown to inhibit DNA topoisomerase and reverse transcriptase, thus disrupting bacterial DNA synthesis and leading to bacterial death [24, 25, 26]. These mechanisms which demonstrated that organic waste-based materials can enhance microbial activity and accelerate the degradation of harmful substances, indicating that bioactive compounds derived from organic waste not only exhibit antibacterial properties but also contribute to broader environmental remediation processes [27].

CONCLUSIONS

The findings of this study demonstrate that ecoenzymes derived from orange and pineapple waste peels exhibit remarkable antibacterial efficacy. The concentration of ecoenzymes exerts a substantial influence on the antibacterial activity against S. aureus and E. coli, as evidenced by the variation in the diameter of the inhibition zone. The study demonstrated that the most effective concentrations of ecoenzymes as antibacterial agents were 75% and 100%, resulting in inhibition zones greater than 20 mm, indicating a high level of antibacterial activity.Consequently, ecoenzymes derived from pineapple and orange peels possess considerable potential for commercialisation as a natural ingredient in household cleaning products. Their high level of antibacterial effectiveness, particularly at the 75% and 100% concentrations, makes them a promising environmentally friendly alternative to conventional cleaning agents. This product has the potential to serve as a safe and sustainable alternative to conventional cleaning products, while also optimising the utilisation of organic waste.

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