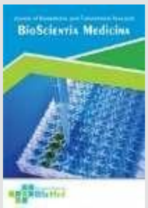

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# Antimicrobial Properties of Eco Enzyme: A Literature Review


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**A B S T R A C T**

**Background**: In surgical medicine, the use of antimicrobials is very important. The use of antimicrobials or antibiotics is used at the time before surgery as prophylaxis, intraoperative, and postoperative. The prevention of some bacterial infections is imperative. The latest research is constantly evolving toward the use of antibiotics, which follows an increasingly resistant antibiotic trend. One of the advancements in the use of antimicrobials is eco enzymes. **Methods:** The literatures reviewed in our study was gathered from PubMed and Google Scholar. The advanced search was conducted on PubMed with the keywords used ("Eco enzyme" AND "antimicrobial" AND "activity" OR "Antibiotics"; "Mechanism" OR "Nature"). The advanced search was also conducted on Google Scholar with all of the words "antimicrobial activity of eco enzyme". We reviewed the articles cited within the literature to broaden the search results. **Results:** Some materials used as eco enzymes in this literature review include *Carica papaya L., Annona muricata L., Azadirachta indica Cymbopogon winterianus, Ananas comosus, citrus aurantium, etc.* Bioactive substances that play a role in microbacterial activity are flavonoids, tannins, saponins, pH, and lactic acids through mechanisms inhibiting nucleic acid synthesis, inhibit cell membrane function, inhibiting energy metabolism of bacteria, and cross-bacterial cell membranes because of the pH gradient, leading to the disruption of cellular metabolic activities of bacterias. **Conclusion:** Eco enzymes have been shown to have antimicrobial abilities.


## 1. Introduction

In surgical medicine, the use of antimicrobials is very important. The use of antimicrobials or antibiotics is used at the time before surgery as prophylaxis, intraoperative, and postoperative. The number of studies of research that supports prophylactic antibiotic therapy as part of standard preoperative care keeps expanding. According to a 2008 study, when administered following total hip and knee replacement, the absolute risk of wound infection is reduced by nearly 80% in comparison to individuals who receive no prophylaxis.[1,2] When a patient is scheduled for surgery with extensive dissections and the potential for increased blood loss, such as when receiving a bone transplant or having artificial implants or foreign objects inserted as part of the procedure, routine prophylactic antibiotic administration is the norm. The prevention of bacterial infections caused by *Staphylococcus aureus,* Epidermidis, *Aerobic streptococci,* and *Anaerobic cocci* makes the administration of this antibiotic treatment imperative.[3]

The latest research is constantly evolving toward the use of antibiotics, which follows an increasingly resistant antibiotic trend. One of the advancements in the use of antimicrobials is eco enzymes. Eco enzyme is a form of liquid extract processed from the fermentation of residues of vegetables and fruits with red sugar or molasses substrate. The principle of the production process of the eco enzyme itself is actually similar to the process of making compost, but water is added as a medium of growth so that the resulting final product is a liquid that is preferred because it is easier to use and has many benefits.[4] The specialty of eco enzyme can generally be made from the skin of fruit and the remains of vegetables, one of which is bananas skin, ananas fruit skin, and cabbage.[5]

The eco enzyme was first developed by Dr. Rasukan Poompanvong from Thailand. Eco enzyme is a multipurpose liquid created by fermenting trash or organic waste with water, brown sugar, or granulated sugar. The production of eco enzymes has a significant impact on the environment on a global and financial scale. It will create and release $O_3$ gas, often known as ozone, during the fermentation process (beginning on day one), which is why it is good for the environment. To lessen greenhouse gases and heavy metals trapped in the atmosphere, this ozone will act underneath the stratosphere. Additionally, $NO_3$ and $CO_2$ gases are created, which the soil requires as plant nutrients.[6]

The use of eco enzymes as antimicrobial properties of fruit peels has been demonstrated against a variety of pathogens, including *Enterococcus faecalis*.[7,8] Fruit peels' antibacterial capabilities are further improved after fermentation as organic materials break down and produce secondary metabolites called bioactive compounds.[7,9] The fermentation approach is preferred over conventional methods for extracting enzymes, organic acids, and phenolic compounds because the latter require expensive solvents, may result in the breakdown of heat-labile compounds, and make it challenging to generate high-purity extracts.[9–11] Additionally, *Enterococcus faecalis* bacteria were unable to develop when an eco enzyme prepared from a blend of orange peel (*Citrus aurantium*) and pineapple peel (*Ananas comosus*) in a 4:6 ratio was used.[10] Due to its antibacterial qualities, the eco enzyme is frequently utilized in Indonesia as a hand sanitizer, disinfection liquid, and bioactive component in liquid soap formulations for domestic usage.[12,13]

Through the action of microbes during fermentation, complex organic compounds are broken down into simpler forms, and beneficial chemicals like antioxidants and antibacterials are produced.[14] Organic acids, phenolic compounds, terpenoids, and alkaloids are some of the secondary metabolites that are produced and have been shown to have antibacterial activities against pathogenic microbes. Polyphenols are recognized as the main naturally occurring antioxidants in diets.[15,16]

However, their bioavailability may be impacted by their being attached to cell walls, glycosylated, or present in polymeric forms, which may compromise their efficiency. However, several metabolic processes take place throughout the fermentation process and contribute to the release or conversion of polyphenols into more active forms.[17] The main problem with preserving food products during storage is oxidation. Thus, the main purpose of antioxidant chemicals is to increase the shelf life of food goods while also having positive effects on health.[16] Based on this, it should be noted that studies on the use of antibiotics should always be conducted and that not much has been done in terms of examining the literature on the use of eco enzymes as antibiotics. Therefore, the goal of this article's authoring is to understand how content activity on an eco enzyme has an antibacterial impact.

## 2. Methods

This study reviewed the literature gathered from two major databases, PubMed and Google Scholar. The advanced search was conducted on PubMed with the keywords used ("Eco enzyme" AND "antimicrobial" AND "activity" OR "Antibiotics"; "Mechanism" OR "Nature"). The advanced search was also conducted on Google Scholar with all of the words "antimicrobial activity of eco enzyme". The showed literatures were further selected based on the publication year, by which we set a 10 years study, starting from 2012 until 2022. This attempt resulted in 320 and 112 literatures gathered from PubMed and Google Scholar, respectively. We also reviewed the articles cited on the references within the article to broaden the search results.

## 3. Results and Discussion

### Eco enzyme

Eco enzyme is an environmentally friendly product. The production of eco enzyme is easy because it is made with simple ingredients. The materials used are also easy to find around. The materials needed to make these products include water, sugar as a source of carbon, and organic garbage from vegetables and fruits. Eco enzymes consist of water, fruit or vegetable residues, and sugar (molasses).[18] In the process of its manufacture, it must be stored in plastic containers, this is to avoid the occurrence of damage to the place when stored in the glass container because the eco enzyme has the result of gas products that can increase the pressure inside the container.[19]

One of the results of the eco enzyme is the content of alcohol and acetic acid. With this content, eco enzymes have the ability to kill bacteria or prevent pathogenic bacteria. From that effect, eco enzymes can be used as antimicrobials. To know how good an eco enzyme is, there are limits on the pH level. A low pH or less than 4 is the criterion for a good eco enzyme. A more acidic pH indicates a higher production of eco enzymes because the low pH is due to the high content of organic acids. Based on this, the eco enzyme is an antimicrobial agent primarily in bacteria *E. coli* because these bacteria cannot live in very acidic conditions.[17,19]

Some materials can be used to make the eco enzymes reviewed in this article, such as papaya peel (*Carica papaya L.*), soursop skin (*Annona muricata L.*), neem leaf (*Azadirachta indica*), lemongrass (*Cymbopogon winterianus*)[20], orange peels (*Citrus aurantium*), black pepper (*Piper nigrum*)[21], Kasturi oranges (*Citrus microcarpa Bunge*), Frangipani sandalwood (*Plumeria alba*), Green leaf extract of tabernaemontana divaricate, Blackberry (*Rubusmi ulfolius)*, Nyamplung (*Calophyllum inophyllum*). Those substances, through the fermentation process that is carried out about 3 days to 3 months, turned out to have a significant effect on suppressing the growth of bacteria.[22] Some also argue that this could be a potential discovery as an alternative to addressing the presence of antibiotic resistance.

### Microorganism involved

In general, bacteria are divided into gram-positive and gram-negative bacteria. Gram-positive bacteria are bacteria that are classified according to their colour on a coloring method that uses violet crystalline dye, which is signed by the thick peptidoglycan cell walls present in gram-positive organisms. This reaction gives a gram-positive organism a blue color when seen under a microscope. Although classically gram-negative organisms have an outer membrane, they have a thinner peptidoglycan layer, which does not resist the blue dye used in the early coloring process. Another piece of information used to distinguish bacteria is their shape. Gram-positive bacteria consist of coconut, basil, or branched filaments.[17]

Gram-negative bacteria have become one of the most significant public health problems in the world due to their high antibiotic resistance. This microorganism has significant clinical importance in hospitals due to its rather severe infectious nature compared to gram-positive bacterial infections. Two large groups of gram-negative groups are Enterobacteriaceae and non-fermenters.[18,19]Some of the pathogens studied in this review of literature is the antimicrobial effects of eco enzymes against bacteria such as *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Prapionibacterium acnes, Methicillin-resistant Staphylococcus aureus, Salmonella Paratyphi, S. pneumoniae* strain D39, *Pseudomonas sp,* and *Bacillus sp.*

### Mechanisms of action of antimicrobial agents

A previous study stated that eco enzymes formed from a combination of papaya peel (*Carica papaya L.*), soursop skin (*Annona muricata L.*), neem leaf (*Azadirachta indica*), and lemongrass (*Cymbopogon winterianus*) with concentrations of 25%, 50%, 75%, 100% proved capable of inhibiting bacteria. This can be judged from the presence of clear zones in the bacterial breeding medium. Eco enzymes with a concentration of 75% and 100% can inhibit the growth of bacteria because the acetate acid contained in it can be used as a disinfectant due to the presence of alcohol and acetic acid in the use of fruit skin as the manufacture of the eco enzyme can also be antibacterial where in this case is the usage of eco enzyme based on the skin of papaya fruit (*Carica papaya L.*), Soursop skin (*Annona muricata L.*), leaves (*Azadirachta indica*) and fragrances (*Cymbopogon winterianus*).[20] The mechanism in the inhibition of bacteria is due to the contents of flavonoids and tannins as bioseptic. The flavonoid content of the eco enzyme fluid will inhibit the synthesis of nucleic acids, inhibit cell membrane function and inhibit energy metabolism, while the contents as antibacterial have activity related to its ability to activate the adhesion of microbial cells, activate enzymes, and interfere with the transport of proteins in the inner layer of the cell.[20,23]

The eco enzyme containing these tannins is in accordance with research by Ramadani et al., which benefits pineapples (*Ananas comosus*). The production of eco enzymes begins with the sorting of raw materials (nanus leather) that are not too dry or rotten. The skin of the pineapple is then washed and disposed of, small-cut, inserted into a plastic container, recycled with red sugar and water, then closed tightly and stored in a cool place, protected from direct sunlight. This storage process is carried out for 3 months, during which a lot of gas is formed from the fermentation reaction, so the plastic container cover needs to be opened periodically to release the gas. In eco enzymes, the most valuable compound to suppress bacterial growth is tannins, which is one of the phenolic chemicals. Tannin has hydroxyl groups and - αβ bonds that are responsible for its antimicrobial properties.[24] Based on Nurliana et al.'s study, these chemical inhibition mechanisms form interactions resulting in a protein-phenolic complex, a non-specific chemical association. The inhibition strength is related to the concentration of phenol content.[25] Low concentrations of tannins form a protein-phenol complex with weak bonds so that it immediately breaks down, damages the cytoplasmic membranes, and causes cell leakage. In addition, in high concentrations, this substance is able to clump with cellular proteins and membrane cytoplasms that eventually cause lisis.[24]

In a study by Rahayu et al. (2021), this tannin compound was also able to inhibit nucleic acid synthesis, inhibit cell membrane function, and inhibit metabolic energy. The mechanism of action of tannins as antibacterials is by inhibiting the reverse enzymes transcriptase and DNA topoisomerase so that bacterial cells cannot form.[23] Tanins have antibacterial activity associated with their ability to activate the adhesion of microbial cells, activate enzymes, and interfere with the transport of proteins in the inner layers of cells.[24]

The other eco-enzyme content that influences the activity of microbes is a combination of pineapple, orange, and papaya. Eco enzymes with a concentration of at least 50% have comparable bacteriostatic effects on *Enterococcus faecalis* with NaOCl 2.5. These results are consistent with previous studies that concluded better antimicrobial effects on endodontic pathogens at higher concentrations of the fruit's eco enzyme content.[26,27] In this eco enzyme, there are hydrolytic enzymes protease and amylase with mechanisms of action destroying the extracellular physical integrity of polymer substances (EPS), the structure of *Enterococcus faecalis*, and causing cell death.[11,28]

The fermentation process of the formation of eco enzymes produces gas. During fermentation, carbohydrates are converted into volatile acids, and in addition, the organic acids present in the waste material are also soluble in the fermented solution because the pH of the waste enzyme is acidic.[29] In the fermentation process, glucose is transformed to produce pyruvate acid. Pyruvate acid in anaerobic conditions will be decomposed by pyruvate decarboxylase into acetaldehyde, and then acetaldehyde is converted by alcohol dehydrogenase into ethanol and carbon dioxide, wherein acetobacter bacteria will convert alcohol to acetylene and water, which will subsequently be converted into acetic acid. After the perfect fermentation process, eco enzymes are formed. Eco enzyme fluids can be used as versatile cleaning fluids such as hand sanitizer and disinfectant.[6,30]

Acetic acid, which is derived from the natural fermentation of the fruit skin, also contributes to its antimicrobial properties. While the concentration of acetic acid indicates that its concentration increases with the longer fermentation period.[27] This is due to the hydrolysis of complex organic compounds into simpler compounds through anaerobic fermentation so that low molecular accumulation of heavy acetic acid occurs. Acetate acid can cross through the membranes of bacterial cells due to the presence of pH gradients, which causes disruption of the cellular metabolic activity of bacteria.[31] The higher osmotic pressure in the bacterial cells also causes the entrance of water and cellular osmolysis.[32]

Based on research by Khameneh (2015) study of the eco enzymes formed from black pepper showed that piperine reduces the MIC of antibiotics by inhibiting the depletion pump and increasing the intracellular concentration of antibacterial agents. The results of the study showed that piperine in liposomal form can inhibit the exhaust pump, and the percentage of ethidium trapped in bromide increased with the presence of piperines.[21]

Another eco enzyme benefit as an antimicrobial agent is the Zno nanoparticles that are obtained from 60 mL of *T. divaricata* leaf extract heated to 80$^0$C and continuously mixed. The solution is then treated with 6 g Zn $(NO_3)_2 6H_2O$ at a temperature of 80$^0$C. The mixture is boiled until a yellow paste is formed. The deposits are heated to 450$^0$C on a stove and stored for 2 hours. The results of these findings were placed in the media and observed. The results of NP ZnO showed higher antibacterial activity against S. aureus and E. coli and lower antibacterial activity towards S. paratyphi compared to standard pharmaceutical formulations. ZnO NP breaks down cell walls, causing membrane damage.[33] This causes the killing of bacteria. ZnO results from green synthesis. NP is very useful for use as an antibacterial active ingredient.[34]

The content of 220D F2 in the eco enzyme formed from the fermentation of *Rubus ulmifolius* is capable of killing preformed pneumococcal biofilms, thereby reducing the population of biofilm cells 6 or 12 hours after treatment by 200 mg/ml from 10% to 1%. The effect of a clear dose-response enabling the killing and release of pre-formed pneumococcal biofilm is observed. Complete detachment of the substrate's biofilm structure was observed after 12 hours of treatment with 200 mg/ml, which correlated well with the number of bacteria showing a 99% decrease in the biofilm biomass. This indicates that 220D-F2 significantly reduces the population of pneumococcal biofilms formed in human pharyngeal cells. Our results show the potential of therapeutic applications of 220D-F2 to damage and kill pneumococcal cells.[35]

**4. Conclusion**

Eco enzymes have been shown to have antimicrobial abilities. The basic ingredients of eco enzymes are diverse such as papaya, bananas, oranges, blackberry, black pepper, and other ingredients. Bioactive content is the most important thing that is useful as antimicrobial activity, such as tannins, saponins, pH, and lactic acid, that will damage the structure of bacteria.

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