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Antibacterial Activity of Ecoenzyme from Garut Orange Peel (*Citrus nobilis var.chrysocarpha*) against *Propionibacterium acne*

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Abstract

Acne is a skin disorder that often occurs in adolescents. Propionibacterium acnes is a bacteria that causes acne. The use of antibacterial substances is very important to prevent and control this bacterial infection. One of the advances in the use of antibacterials comes from the use of ecoenzyme. The process of managing organic waste with ecoenzyme can be an effective and environmentally friendly waste management strategy. Garut orange with the scientific name *Citrus nobilis var. chrysocarpa* is a local wisdom fruit. Orange peel which is a waste that increases in quantity when the harvest season arrives can be used in the manufacture of ecoenzyme. This study aims to evaluate the antibacterial ability of ecoenzyme from garut orange peel against Propionibacterium acnes bacteria. The fermentation time for ecoenzyme production varied from 30, 60 and 90 days.. The method for the eco enzyme antibacterial test uses the disc diffusion method. Three variations in eco enzyme concentration used to inhibit the growth of isolated bacteria are 33%, 66% and 100%. Ecoenzyme from Garut orange peel shows antibacterial activity against *Propionibacterium acne*. Antibacterial activity shows an increase with increasing fermentation time of ecoenzyme.

Keyword: Antibacterial, Ecoenzyme, Orange peel, *Propionibacterium acnes*.

INTRODUCTION

Acne (acne vulgaris) is a common skin disorder, especially in adolescents and young adults (Lynn et al., 2016). This disease is often caused by inflammation and infection of the pilosebaceous unit by bacteria such as Propionibacterium acnes. This infection is triggered by factors such as hormones, stress, and skin hygiene, resulting in increased sebum production that creates an ideal environment for the growth of pathogenic bacteria (Kim & Kim, 2024). As a result, acne can have a negative impact on the quality of life of sufferers, both physically and

psychologically(Zhou et al., 2023).

Most of the acne medications currently available are based on synthetic antibiotics such as tetracycline, clindamycin, and erythromycin. However, long-term use of these antibiotics can cause bacterial resistance that has the potential to reduce the effectiveness of therapy(Reynolds et al., 2024). Therefore, alternative treatments based on natural ingredients are needed that are safer, more effective, and environmentally friendly to treat acne. One approach that has attracted attention is the use of natural antibacterials containing bioactive compounds (Cristani & Micale, 2024)

One of the advances in the use of antibacterials comes from the use of ecoenzyme (Tallei et al., 2023). Ecoenzyme are liquid extracts from the processing of fermented vegetable and fruit waste. The results of the fermentation of fruit skin, brown sugar, and water are known as ecoenzyme. The fermentation process takes place over a certain period in an environmentally friendly manner involving the collection of kitchen waste such as fruit peels, vegetable scraps, and other organic materials (Novianti & Muliarta, 2021).

Indonesia as an agricultural country with abundant vegetable and fruit production. The large number of vegetable and fruit products also contributes to the increase in the amount of organic waste (Muhammad et al., n.d.). Data from the Ministry of Environment and Forestry in 2022, Indonesia contributed 68.5 million tons of waste. As much as 31.65% of this waste comes from household waste (Hanifah et al., 2022). Optimal waste management is needed so that this vegetable and fruit waste can be utilized and does not pollute the environment. The process of managing organic waste with ecoenzyme can be an effective waste management strategy. Organic and non-organic waste is a threat to the environment if not managed properly (Verma et al., 2019).

The fermentation process in ecoenzyme produces a solution containing beneficial microbes, enzymes, and organic acids. The involvement of organisms in the fermentation process plays a role in breaking down complex organic compounds into simpler forms and producing bioactive compounds, such as antibacterials and antioxidants (Sharma et al., 2020). Bioactive compounds are secondary metabolites including organic acids, phenolic compounds, terpenoids, and alkaloids, which have been found to have antimicrobial properties against pathogenic

microorganisms(Liu et al., 2023). Polyphenols as natural antioxidants, during the fermentation process are converted into more active forms (Verni & Rizzello, 2023).

Ecoenzyme solutions have many advantages, including for agriculture as liquid organic fertilizers and natural pesticides (Novianto, 2022), disinfectants (Rahayu et al., 2021), organic soaps (Latifah et al., 2022), floor and bathroom cleaners (Istanti & Utami, 2022). Ecoenzyme solutions can be used as cleaning solutions because of the enzymes they contain, which are able to break down proteins and fats (Rahayu et al., 2021)

Garut oranges with the scientific name *Citrus nobilis var. chrysocarpa* come from the Garut area, West Java, Indonesia (Utami et al., 2015). This orange farming provides economic contributions to local farmers (Lungguh Perceka & Rusyani, 2025). This orange has certain characteristics and specialties that distinguish it from other types of oranges. Garut oranges are known to have a sweet and fresh taste with a distinctive aroma. Garut orange peel is usually bright orange. This orange can be round or slightly flat, depending on the variety. Garut orange is often used as a fresh fruit that is delicious to eat directly. In addition to the juice can also be made into a fresh drink, orange peel is often used in various products including the manufacture of ecoenzyme or other natural ingredients (Aulia & Handayani, 2022).

Given the facts explained, this study aims to evaluate the antibacterial ability of ecoenzyme from garut orange peel. Garut orange peel is a fruit waste that is often found when the harvest season arrives. With this study, it is hoped that ecoenzyme will become a technology for the development and production of antibacterial substances Propionibacterium acnes by utilizing waste from the local wisdom of Garut city.

RESEARCH METHOD

The research was conducted with experiments in the Microbiology Laboratory, Campus 1 STIKes Karsa Husada Garut

Tools and materials

The tools needed in this study Petri Dish, Autoclave, Laminar Air Flow (LAF), incubator, Vernier Caliper, Disk Diffusion Paper, Vortex Mixer, Micropipette and Tips, Test Tube, Erlenmeyer, Bunsen Burner, micropipette, spreader, Sterile Cotton Swab, plastic bottle. Materials needed in the study nutrient broth (NB), muller hinton agar (MHA), distilled water, 70% alcohol, 0.9% physiological NaCl, brown sugar, garut

orange peel

Ecoenzyme Preparation

All Garut orange peels *Citrus nobilis var. chrysocarpa* are washed and then cut into about 2 × 2 cm, then placed in a container. A total of 0.5 kg of brown sugar is cut into pieces, boiled in 5 liters of clean water, and then cooled. Next, the sugar solution was added to the container containing 1.5 kg of orange peel pieces. The container was tightly sealed and fermented for 90 days. After ten days of fermentation, the lid was opened and the fermentation liquid was stirred evenly. The container was then sealed with tape and left to ferment for three months. Samples were obtained from ecoenzyme that had been fermented for 30, 60 and 90 days. Measurements taken at 30, 60 and 90 days during the fermentation process can provide valuable information regarding the short-term and long-term effects of fermentation on microbial activity.

Preparation of Ecoenzyme concentration

Ecoenzyme with a concentration of 100% after fermentation was sterilely filtered and then diluted to concentrations of 66% and 33% respectively for antimicrobial testing against Propionibacterium acnes bacteria. The 0% concentration control used distilled water

Nutrient Broth Media Preparation

To make nutrient broth, weigh the ingredients according to the recipe size, dissolve with distilled water. Stir until the media is homogeneous. After that, put 5 ml of each into a reaction tube. Sterilize the solution in an autoclave at 121°C for 15 minutes.

Muller Hinton Agar media Preparation

MH media is made by weighing all ingredients according to the recipe measurements. Dissolve the media with distilled water in an Erlenmeyer flask. Stir until homogeneous. Heat the mixture using a hot plate to completely dissolve the agar. Stir slowly until the solution becomes clear. Sterilize the solution in an autoclave at 121°C for 15 minutes at a pressure of 1 atm. After the autoclave is complete, allow the agar solution to cool slightly (around 45–50°C) so as not to cause condensation in the petri dish. Pour the solution into sterile petri dishes with sufficient volume to form a layer (around 20–25 mL per dish). Allow the media to solidify at room temperature. After solidifying, store the MHA media at 4°C if not used immediately. Close the petri dish tightly to prevent contamination.

Bacterial Strains Preparation

The inoculum was prepared based on the Clinical and Laboratory Standards Institute (CLSI) protocol. Propionibacterium

acnes bacteria were cultured in nutrient broth liquid media and incubated at 37◦C for 24 hours. The density of bacterial cells was then adjusted to 0.5 McFarland Standard by dilution using physiological NaCl.

Determination of Ecoenzyme Activity by Diffusion Method

The determination of antimicrobial activity of citrus waste ecoenzyme was carried out by the disc diffusion method. In this method, ecoenzyme with several concentrations of 0, 33, 66 and 100% obtained will be absorbed by a disc (paper disk) and placed on an agar medium that has been smeared with bacteria. After that, incubation was carried out for 24 hours at 37 ºC. Antimicrobial activity was measured by the presence of a clear zone around the disc.

RESULT AND DISCUSSION

Orange peel ecoenzyme was made by fermenting orange peel *Citrus Nobilis var. chrysocarpha*, sugar and water in a ratio of 3:1:10. Evaluation of the antibacterial ability of the garut orange peel ecoenzyme was carried out by the diffusion method. The concentration of the ecoenzyme was varied with sterile aquadest diluent at concentrations of 33%, 66% and 100%. Negative control used sterile aquadest with an ecoenzyme concentration of 0%. Antimicrobial activity was seen from the clear zone around the disc that was not overgrown by bacteria. The results of the antimicrobial activity test of the Garut orange peel ecoenzyme on bacteria are shown in Figure 1. The size of the measured inhibition zone is presented in Table 1.

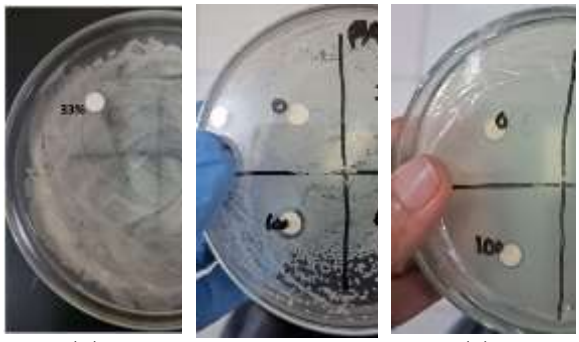

Figure 1. Results of the antimicrobial activity test of the ecoenzyme of garut orange peel after fermentation for 30 days (a) 60 days (b) and 90 days (c) with concentrations of 33%, 66% and 100% on *Propionibacterium acne* bacteria.

From the results of the antibacterial activity test of the ecoenzyme of garut orange peel waste, it shows a clear zone around the disc. This shows that the growth of Propionibacterium acnes bacteria is inhibited by the presence of ecoenzyme. Bioactive compounds formed during the fermentation of garut orange peel ecoenzyme provide an

inhibitory effect on microbial growth. This is in line with research on ecoenzyme made from pineapple and papaya peels, which show the effectiveness of inhibiting *Staphylococcus aureus* and *Propionibacterium acnes* at concentrations of 50%, 75% and 100% (Ramadani et al., 2022).

Table 1. Antibacterial activity of orange peel ecoenzyme against *Propionibacterium acnes* bacteria.

Ecoenzyme concentration (%)	**Inhibition zone (mm) on day of fermentation**		
	30	60	90
33	6.5	6	10
66	6.5	7	11
100	6.5	10	9

Ecoenzyme from orange peel *Citrus nobilis var.chrysocarpha* with a fermentation time of 30 days showed a very small ability to inhibit the growth of Propionibacterium acnes. After 60 days of fermentation, the antibacterial ability was increasingly apparent at concentrations of 66% and 100%. Antibacterial activity increased with the concentration of ecoenzyme. The inhibition zone of *Propionibacterium acnes* bacterial growth was increasingly visible after 60 days of fermentation at an ecoenzyme concentration of 33% and its diameter increased at a concentration of 66%, but decreased at a concentration of 100%. The optimum concentration of ecoenzyme in inhibiting bacteria in 90-day fermentation was at a concentration of 66%.

The length of the fermentation process in making ecoenzyme determines its antibacterial effectiveness. The results of the research carried out the fermentation process was carried out for 30, 60 and 90 days. Within two months, the fermentation process was quite effective in producing ecoenzyme with antibacterial ability. Literature studies show that long-term fermentation for 3 months or around 90-100 days usually produces ecoenzyme that are richer in active compounds, which provide stronger antibacterial effects (Mavani et al., 2020).

The mechanism of inhibition of Propionibacterium acne bacteria by garut orange peel ecoenzyme can be associated with the antimicrobial effects of compounds formed during fermentation. Phenolics are known to inhibit bacterial enzyme activity, disrupt cell membrane permeability, and cause leakage of cell contents which ultimately stops bacterial growth (Ledo et al., 2024). The mechanism of inhibition by orange peel

ecoenzyme can also be associated with the presence of compounds such as organic acids (acetic acid, citric acid) and are formed during fermentation. These compounds are known to have antibacterial activity by damaging bacterial cell membranes, inhibiting bacterial metabolism, causing bacterial cell death (Das et al., 2024).

The advantages of garut orange peel ecoenzyme as a natural antibacterial agent where the production process is simple and environmentally friendly, which utilizes typical fruit peel waste in the city of Garut. In addition, its use as an alternative natural antibacterial can reduce dependence on synthetic antibiotics which often cause resistance in pathogenic bacteria (Neupane & Khadka, 2019). Several other studies have shown that ecoenzyme with natural acid composition and organic compounds can provide antibacterial effects without adverse side effects (Oviantari et al., 2023).

The potential for developing ecoenzyme-based products not only provides antibacterial benefits but is also environmentally friendly because it uses organic waste raw materials. The results of the study showed that the ecoenzyme of garut orange peel showed the activity of Propionibacterium acne bacteria which has high potential to be applied in natural skin care products for acne treatment, especially on sensitive skin that is prone to irritation due to the use of synthetic antibiotics. Further development can be done by optimizing the concentration and formulation so that the ecoenzyme has a wider spectrum of activity against various pathogenic bacteria such as Propionibacterium acne. Natural organic compounds in ecoenzyme, such as citric acid and limonene from oranges, can act as antiseptics and anti-inflammatories, which help reduce inflammation in acne (Ramadani et al., 2022).

CONCLUSION

Ecoenzyme from Garut Orange Peel (*Citrus nobilis var.chrysocarpha*) shows antibacterial activity against Propionibacterium acne. Antibacterial activity shows an increase with increasing fermentation time of ecoenzyme

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