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ECO-ENZYME BASED ON HORTICULTURAL WASTE'S WITH VARYING CONCENTRATION ON THE ACTIVITY OF CANDIDA ALBICANS FUNGUS IN VITRO

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Abstract

This study aims to evaluate the potential of eco-enzyme (EE) derived from horticultural waste as an antifungal agent against Candida albicans, a major pathogen responsible for infections in both humans and plants. Eco-enzyme is a product of fermentation that involves using organic waste, such as fruit and vegetable peels, which are often discarded and contribute to environmental pollution. In this research, eco-enzyme was prepared using a variety of horticultural waste, including pineapple, orange, papaya, and mango peels. The eco-enzyme was then tested at various concentrations (1:0, 1:10, 1:50, and 1:100) to assess its antifungal activity against Candida albicans using the in-vitro disk diffusion method. The results indicated that eco-enzyme, particularly at the 1:50 dilution, demonstrated significant antifungal activity, with a clear inhibition zone observed around the disc. The presence of bioactive compounds such as flavonoids, tannins, and saponins in the eco-enzyme is believed to be responsible for its ability to inhibit fungal growth by disrupting the cell membrane structure and metabolic functions of the pathogen. These findings suggest that eco-enzyme can serve as an effective natural alternative to conventional chemical antifungal agents, offering a more sustainable and eco-friendly solution for managing fungal infections. Furthermore, this study emphasizes the importance of utilizing agricultural waste for eco-enzyme production, providing an innovative way to address the global challenge of organic waste disposal while promoting sustainable agricultural practices. The research contributes to the growing body of knowledge on the benefits of eco-enzyme in both agricultural and public health applications, offering promising possibilities for its wider use in fungal disease management

Keywords: eco-enzyme; Candida_albicans; antifungal; organic_waste; natural_disinfectant

INTRODUCTION

Fruit and vegetable production in Indonesia generates more than 30 million tons of horticultural waste annually. If this waste is not properly managed, it can lead to environmental pollution. On the other hand, Candida albicans is an opportunistic fungus that causes candidiasis, with a global prevalence of over 250,000 cases annually and a mortality rate of 20–40% (CDC, 2020). The use of synthetic antifungal drugs such as azoles can lead to resistance and side effects. Therefore, safer and more environmentally friendly natural antifungal alternatives are needed. Various studies have demonstrated the antibacterial potential of eco-enzymes (Mavani et al., 2020; R.amadani et al., 2022; Rusdianasari et al., 2021); however, research on their antifungal activity remains limited (Lubis, Damayanti, et al., 2024). Theoretically, eco-enzymes contain organic acids, phenolics, flavonoids, and terpenoids that can damage fungal cell membranes and inhibit growth. However, secondary metabolites from plants have several properties as medicinal plants, such as from the Turmeric plant (Curcuma longa L.) which can inhibit the growth of Staphylococcus aureus (Astari et al., 2021). Also, moringa leaves and green tea and their combination act as antibacterial agents that can fight acne (Wulandari et al., 2020). Previous studies have also demonstrated that eco-enzymes derived from fruit peels exhibit effective antibacterial activity; however, research on their antifungal activity, particularly against Candida albicans, remains limited (Rusdianasari et al., 2021). Pineapples, oranges, and noni fruits are rich in bioactive compounds that support antifungal activity. Research conducted by Mavani et al. (2020) found that eco-enzymes derived from various types of organic waste, including pineapple, orange, and papaya peels, exhibit antibacterial properties against Enterococcus faecalis and Staphylococcus aureus (Mavani et al., 2020), while research by Ginting & Prayitno (2022) shows that eco-enzymes can be used as disinfectants for livestock pen sanitation (Ginting et al., 2021) and as an antibacterial against Staphylococcus aureus (Ginting & Prayitno, 2022). Meanwhile, research by Lubis et al. (2024) revealed that eco-enzymes derived from certain fruit waste are effective in inhibiting the growth of the fungus Fusarium oxysporum in horticultural plants (Lubis, Tarigan, et al., 2024). The study demonstrated that eco-enzymes derived from pineapple, orange, and papaya waste effectively inhibited the growth of the fungus Fusarium oxysporum, which causes wilt disease in chili and tomato plants. This study shows that eco-enzymes are not only useful as natural fertilizers and pesticides, but can also be used to control diseases caused by pathogenic fungi. In addition, eco-enzymes also have the potential to replace the use of chemical pesticides that are harmful to the environment. Applying eco-enzymes to plants can not only increase plant resistance to fungal diseases but can also improve soil quality and increase overall agricultural yields (Lubis, Wasito, et al., 2024). This makes eco-enzymes an environmentally friendly and effective alternative in sustainable agricultural systems.

From several descriptions of research results, it is seen that eco-enzymes can act as antibacterial agents. However, the use of eco-enzymes as antifungals, particularly against Candida albicans, remains very limited. Candida albicans is a pathogenic fungus that can cause infections in the skin and other human body systems, as well as being the cause of diseases in horticultural plants, such as wilt disease. This fungal infection is a significant concern in the realm of health, particularly in tropical countries with high humidity, which provide an ideal habitat for fungal growth. This study aims to evaluate the potential of eco-enzymes derived from horticultural organic waste in inhibiting the growth of Candida albicans. Various concentrations of eco-enzymes made from organic waste commonly produced in agriculture, such as pineapple, orange, papaya, and mango peels, were tested to determine which concentration was most effective as a natural disinfectant and could inhibit the growth of the fungus.

LITERATURE REVIEW

Organic waste, particularly from agricultural and horticultural products, is a type of waste whose volume continues to increase in both rural and urban areas. Poor management of this organic waste can lead to environmental pollution, including air, soil, and water pollution. One way to mitigate the negative impacts of organic waste is to process it into useful products, such as liquid organic fertilizer (LOF) and eco-enzymes (EE) (Yulistia & Chimayati, 2021). The use of POC has been widely used to increase plant production, including POC from cow dung can increase the number of shallots (Erianti et al., 2024), the treatment of GDM commercial liquid organic fertilizer concentration has a real effect on the growth and yield of shallots (Cahyati et al., 2024), also at a dose of 400 g/polybag cow dung fertilizer increases plant height and number of leaves in purple eggplant plants (Nabila et al., 2025), Liquid Organic Fertilizer derived from cow dung in the type of planting media will affect parameters in the form of number of leaves, leaf area, plant height, root decay ratio, and fresh weight of lettuce plants (Siregar et al., 2025) Another use of organic waste is to process it into eco-enzyme products (Apriliansyah & Wibisono, 2022). Eco-enzyme (EE) is the result of fermenting organic waste with molasses and water. Eco-enzyme was first introduced by Dr. Rosukon Poompanvong from Thailand as a solution to reduce the daily production of organic waste. This product is produced through a fermentation process using basic ingredients, including fruits, vegetables, and brown sugar. Eco-enzyme is the result of fermenting organic waste that can be used for various purposes. Eco-enzyme has various benefits, including as an organic fertilizer, a botanical insecticide for cocoa plants, as a natural pesticide to inhibit the growth and mortality of S. litura (Megasari et al., 2025), a cleaning fluid , an antimicrobial (Permatananda et al., 2023), and an environmentally friendly natural disinfectant (Novianti & Muliarta, 2021);(Lubis et al., 2025)

RESEARCH METHODS

This research was conducted at two different locations to accommodate the type of analysis performed. The first stage, the preparation of research materials, was conducted at the Agrotechnology Study Program Laboratory, Universitas Pembangunan Panca Budi. The second stage, the antifungal activity testing, was conducted at Microbiology Laboratory, Universitas Sumatera Utara, because this laboratory has facilities that support testing of pathogenic microbes such as Candida albicans.

Research Materials

The main ingredients used consist of various types of local organic waste obtained from the surrounding environment. These include pineapple, orange, papaya, starfruit, quinoa, mango, banana stems, and noni. Molasses is also used as a carbon source for fermentation, water as a solvent, and Candida albicans cultures as test microbes.

The selection of local organic materials is based on their abundant availability, their biodegradability, and their potential secondary metabolite content that can act as antimicrobial compounds.

Eco-Enzyme Production

Eco-enzyme is formulated based on the composition of ingredients: molasses: water with a ratio of 3:1:10. Organic materials are chopped into small pieces to accelerate the degradation process, then mixed with molasses and water according to the specified ratio. The mixture is placed in a tightly closed plastic or glass container to prevent contamination from the outside environment. The fermentation process lasts for 100 days at room temperature. During fermentation, the container is opened periodically to release gases resulting from microbial metabolism. After 100 days, the fermented liquid is filtered to separate the solids from the filtrate, which is then used as the test eco-enzyme solution (Figure 1).

Antifungal Activity Test

The antifungal activity of eco-enzyme was tested using the disc diffusion method. In the initial stage, Candida albicans culture was propagated in a suitable agar medium until it reached an adequate cell density. Next, the microbial suspension was evenly inoculated on the surface of the agar medium in a petri dish. After that, sterile paper discs were dipped into eco-enzyme solutions with various predetermined concentrations, namely 1:0 (positive control without dilution), 1:10, 1:50, 1:100, and 1:150. The discs containing the solution were then placed on the surface of the agar medium that had been inoculated with C. albicans .

The petri dishes were then incubated for 48 hours at 37 °C. After incubation, the clear zone around the disc, indicating fungal growth inhibition, was measured using a digital ruler to obtain the diameter of the inhibition zone with high accuracy.

Data analysis

The results of the inhibition zone diameter measurements are interpreted based on categories according to Davis & Stout (1971), namely: weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm) (Davis & Stout, 1971) . The measurement data were then tabulated and analyzed descriptively to compare the effectiveness of various eco-enzyme concentrations on the growth of C. albicans. All research procedures were conducted in accordance with laboratory safety standards, particularly when handling pathogenic microbes. Instruments used, including pipettes, discs, and media, were sterilized prior to use to prevent cross-contamination. Furthermore, each testing step was repeated at least three times to ensure the validity of the results and minimize experimental error.

With this approach, the research is expected to provide a comprehensive picture of the potential of local organic waste-based eco-enzymes as natural antifungal agents against Candida albicans.

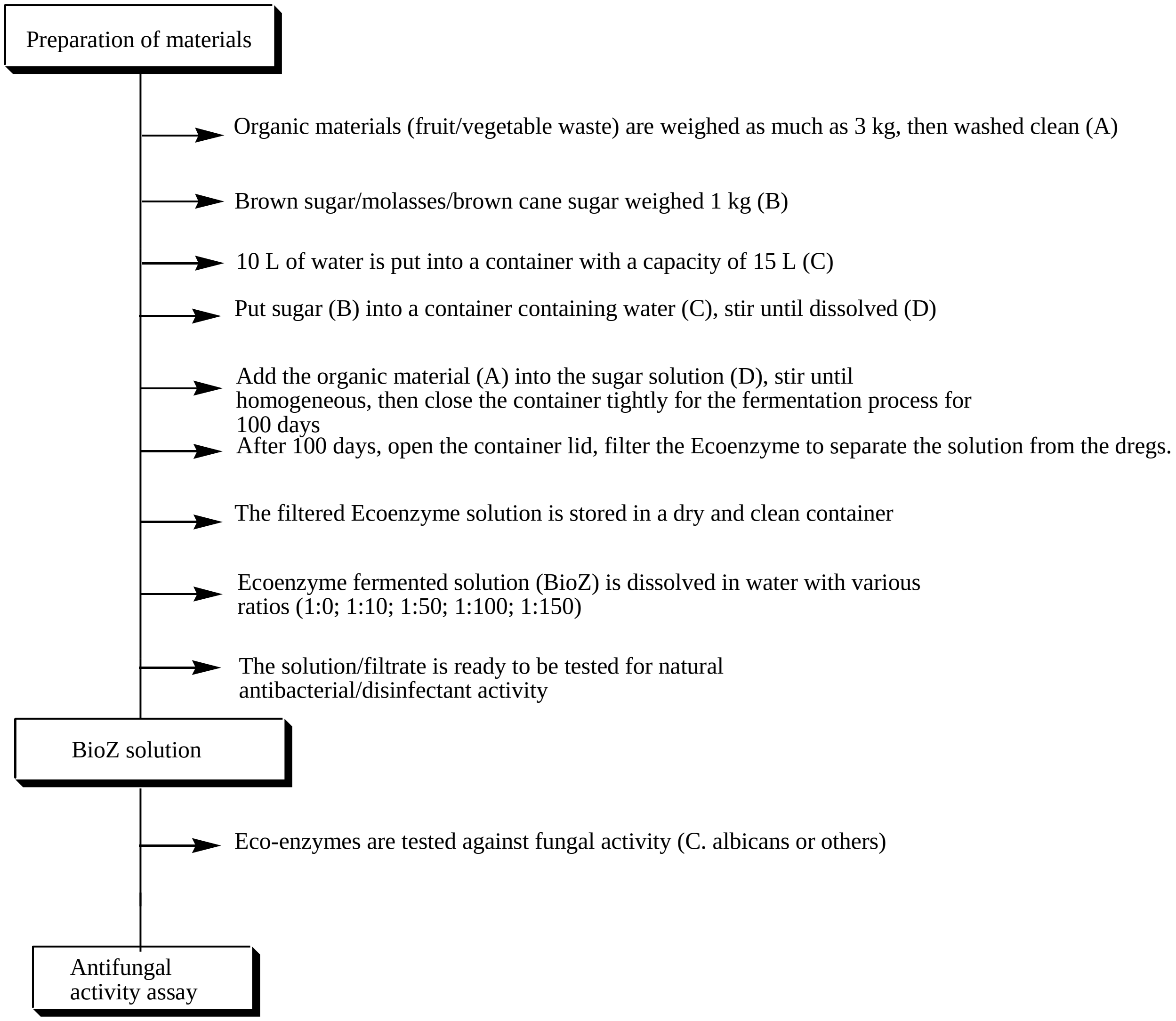

Figure 1. Flow chart of ecoenzyme production research

RESULTS AND DISCUSSION
RESULTS

The results of the ecoenzyme (Bioz1 and Bioz2) activity assay as an antifungal are shown in Figure 2.

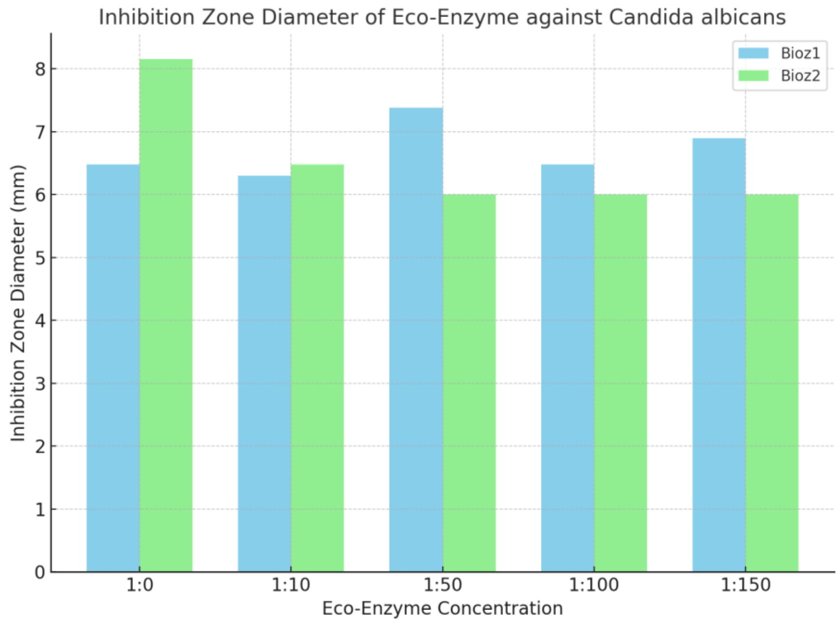

Figure 2. Diameter of inhibition zone due to variation in Eco-enzyme concentration

DISCUSSION

Based on the Davis & Stout (1971) classification, the inhibition zone in the range of 5–10 mm is included in the moderate category , so the eco-enzyme studied can be said to have potential as a fungal growth inhibitor. Although it does not reach the strong category (>10 mm), this result is still relevant considering that the eco-enzyme is made from organic waste materials with a simple fermentation process, so its sustainability is very high.

The difference in antifungal response between Bioz1 and Bioz2 suggests that the composition of the ingredients plays a significant role in the effectiveness of the eco-enzyme. Bioz1 performed best at a concentration of 1:50, while Bioz2 was more effective at a concentration of 1:0 (figure 2). This can be explained by several factors:

1. Secondary metabolite content of organic materials
   Pineapple and orange fruits contained in the Bioz1 formulation are known to be rich in citric acid and phenolic compounds which have the ability to disrupt the integrity of microbial cell membranes (Yong et al., 2019). Secondary metabolite compounds are known to have properties including anti-inflammatory activity, such as in ginger and lemongrass extracts (Faradiba et al., 2024)
   The noni fruit (Morinda citrifolia) found in both formulations also contains iridoid and flavonoid compounds which act as antifungal agents.
2. The effect of concentration on effectiveness
   Too high a concentration (1:0) sometimes does not show the best results because of the possibility of the presence of growth-inhibiting compounds which are actually toxic to other active metabolites.
   A concentration of 1:50 in Bioz1 provides an optimal balance between active metabolite levels and diffusion capacity into the agar medium, resulting in the largest inhibition zone.
3. Stability of results in Bioz2
   The relatively consistent measurement results of Bioz2 at concentrations of 1:10 to 1:150 indicate that the active compound in this formulation may have a lower level of stability or may only be effective under certain conditions.

In general, the inhibition zone formed in this study ranged from 6.0 to 8.16 mm , which is considered moderate. This indicates that eco-enzyme is capable of inhibiting the growth of Candida albicans , but has not yet demonstrated its potential as a very potent antifungal agent. This interpretation is consistent with the literature which states that organic fermentation products usually produce secondary metabolite compounds in limited quantities, so that their effects are not as strong as synthetic antibiotics. (Ramadani et al., 2022) . However, the advantages of eco-enzymes lie in their environmentally friendly nature, low cost, and the sustainability of their raw materials.

Some bioactive compounds that may contribute to the antifungal activity of eco-enzyme include:

a) Citric acid and other organic acids from pineapple, orange, and star fruit can lower the pH of the medium, thereby inhibiting fungal growth.
b) Flavonoids and phenolics from papaya, mango, and quinoa are able to damage fungal cell membranes through oxidative mechanisms.
c) Iridoids and scopoletin from noni act as antimicrobial agents with an enzyme inhibition mechanism.
d) Lignocellulosic components are broken down from banana stems which during fermentation can produce simple phenolic compounds.

This combination of bioactive compounds has the potential to provide a synergistic effect in inhibiting fungal growth, although its effectiveness still needs to be improved through optimization of the fermentation process and material formulation.
The results of this study align with those of Yong et al. (2019), who reported that phenolic-rich tropical fruit extracts possess significant antimicrobial activity. Similarly, Ramadani et al. (2022) demonstrated that the use of horticultural waste-based eco-enzymes can inhibit the growth of several pathogenic microbes.
However, compared to pure extracts or synthetic compounds, eco-enzyme activity is relatively lower. This is understandable because eco-enzymes are the result of fermenting a mixture of organic materials without a purification process, resulting in lower concentrations of active compounds.

1. Sustainable raw materials: using abundant local organic waste.
2. Environmentally friendly: does not produce harmful chemical residues.
3. Low cost: the simple fermentation process can be carried out by the community.
4. Wide application potential: as a biofungicide for organic farming and as a non-clinical fungal control agent.

1. Antifungal activity is still moderate: it has not yet reached the strong category.
2. Variability of results: differences in ingredient composition result in different effectiveness.
3. Lack of standardization: traditional fermentation processes are susceptible to environmental variations.
4. Toxicity not tested evaluation of impact on non-target cells is required.

To increase the potential of eco-enzymes as antifungal agents, several research directions can be pursued:

a) Optimization of organic material formulation to increase active metabolite content.
b) Purification of bioactive compounds from eco-enzymes to identify the main components involved.
c) Activity test against other pathogenic fungal strains to determine its spectrum of action.
d) Safety evaluation of eco-enzyme use in plants, animals and humans.
e) Application of controlled fermentation technology to produce more consistent products.

The results of this research have practical implications for the development of natural biofungicides. By utilizing local organic waste, communities can produce value-added products useful in sustainable agriculture. Furthermore, eco-enzymes can be an alternative to synthetic fungicides, which often produce hazardous residues. While the results of this study are promising, there are several critical caveats. First, the inhibition zone obtained is still relatively small compared to commercial antifungal agents, thus limiting its application in the medical field. Second, the variation in results between Bioz1 and Bioz2 indicates the need for formulation standardization for more measurable effectiveness. Third, the mechanism of action of eco-enzyme against Candida albicans has not been fully elucidated, so further research into the molecular mechanisms is needed.

CONCLUSION

This study shows that eco-enzymes based on local organic waste have moderate antifungal activity against Candida albicans . Bioz1 was most effective at a concentration of 1:50 (7.38 mm), while Bioz2 was best at 1:0 (8.16 mm). These results confirm that the composition of the ingredients affects effectiveness, so optimization of formulation and fermentation is needed to increase the potential of eco-enzymes as environmentally friendly biofungicides. Also, this study is still limited to in vitro tests against Candida albicans . Therefore, Further research is recommended with optimization of eco-enzyme formulation, testing at wider concentrations, and identification of dominant active compounds , and in vivo testing and toxicity testing are needed to ensure the safety and effectiveness of eco-enzymes in agricultural and health applications .

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