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Effectiveness of Ecoenzyme from Eucalyptus Leaves Produced by MSMEs Lamongan as A Disinfectant

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ABSTRACT

Hand washing with running water for at least 20 seconds is one of the most effective methods for preventing the transmission of infection-causing microorganisms. However, the increasing use of alcohol-based hand disinfectants as a practical alternative has raised concerns because of adverse effects such as skin irritation, dryness, and dehydration. Consequently, plant-based disinfectants with fewer side effects have attracted growing attention. One potential material is eucalyptus (*Melaleuca leucadendra*), whose leaves can be fermented into ecoenzyme containing bioactive compounds with reported antibacterial properties. This study aimed to evaluate the antibacterial effectiveness of eucalyptus leaf ecoenzyme produced by Lamongan MSMEs as a hand disinfectant. A simple experimental study with a comparative design was conducted using bacterial swab samples collected from the fingers of three randomly selected Universitas Airlangga students. Antibacterial activity was assessed by comparing bacterial colony counts on Mueller–Hinton agar after treatment with eucalyptus leaf ecoenzyme at concentrations ranging from 10% to 100%. The results showed that none of the tested concentrations significantly reduced bacterial colony counts, indicating limited antibacterial activity against bacteria on the hand surface. It can be concluded that eucalyptus leaf ecoenzyme produced by Lamongan MSMEs at concentrations of 10%–100% does not demonstrate significant antibacterial effectiveness. These findings suggest that optimization of the fermentation process, raw-material composition, and organic-acid content is necessary before the ecoenzyme can be recommended as an alternative organic hand disinfectant.

Keywords: Ekoenzim, eucalyptus, disinfectant,green product, recycling, health risk.

INTRODUCTION

Hand washing is one of the three pillars of health development. Washing your hands properly and properly can kill the germs that cause infections in your hands (Suswati & Maulida, 2020). Hand hygiene needs to be maintained because it often comes into direct contact with the mouth, nose, and surrounding objects. Recommendations from *Centres for Disease Control and Prevention* (CDC) Hand washing should be done under running water for 20 seconds before holding the nose, before eating, after sneezing and coughing. If water and soap are not available, it is still recommended to use an antiseptic containing 70% alcohol (Utami, 2022; Ratmaja et al., 2023).According to research conducted by (Luby et al., 2009) Washing hands with soap properly can reduce the incidence of diarrhea by 31% and reduce the incidence of upper respiratory tract infections (ARI) by 21%. Hand washing can not only reduce, but can prevent diarrhea by up to 50% and ISPA by up to 45% (Sunardi & Ruhyanuddin, 2017). The use of alcohol as a disinfectant instead of soap is increasingly used as human busyness increases (Desiyanto & Djannah, 2013).

The relatively small form of antiseptic so that it is easy to carry is also the main consideration for the increasing use of alcohol-based antiseptics for hand washing. Alcohol with a concentration of 70% has been proven to be able to kill germs effectively because it can denatturize and coagulate bacterial proteins (Andriyansyah et al., 2022).

The disadvantage of alcohol as the main ingredient of disinfectant is that it has negative side effects for humans. Continuous use of alcohol on the hands can cause irritation, dry skin, and dehydration (Lachenmeier, 2022). To reduce the side effects of alcohol use, organic-based disinfectants are needed that are more environmentally friendly and have fewer side effects on humans (Dharmawanti et al., 2021). One of the organic materials that can be used is eucalyptus plants *(Go to leucadendra).* The leaves of eucalyptus plants have seven active compounds that have broad-spectrum antibacterial effects. The way these compounds work is by inhibiting the process of forming cell walls, destroying genetic material, and inhibiting the action of enzymes in bacteria (Joen, 2020). To get the benefits of eucalyptus leaf substances, eucalyptus leaves can be processed by a fermentation process or commonly referred to as ecoenzymes. Ekeonzim has active compounds that can kill bacteria so that it can be used as a disinfectant (Sarlinda & Nurkhoiriyah, 2023). Lamongan is a district in East Java Province that is a producer of eucalyptus plants (*Let's go to Leucadendra*). The geographical location of Lamongan which is close to the research location and the abundance of eucalyptus leaf commodities, can be an ideal consideration as the main source of research materials. The potential of the abundance of eucalyptus leaves with the right combination of cutting, will produce products that are of high value and have the potential to be developed.

This study aims to prove the effect of ecoenzyme disinfectant of eucalyptus leaves produced by Lamongan MSMEs as an effort to replace chemical-based disinfectants that have side effects on humans. Based on research (Rijal et al., 2021) which shows that the ecoenzyme of Maluku eucalyptus leaves can inhibit the growth of bacteria. Meanwhile, the research on eucalyptus leaf ecoenzymes produced by Lamongan has never been researched before. Therefore, the researcher proposed this study to determine the effect of eucalyptus leaf ecoenzyme disinfectant produced by Lamongan MSMEs.To clarify the scientific problem underlying this study, the following points are emphasized. Socially, the growing preference for alcohol-based disinfectants over handwashing with running water reflects the increasing busyness of modern life, yet inadequate hand hygiene remains associated with a substantial disease burden: handwashing with soap has been reported to reduce the incidence of diarrhea by 31% and acute respiratory infections by 21% (Luby et al., 2009b) and to prevent diarrhea by up to 50% and respiratory infections by up to 45%). Although alcohol-based disinfectants are effective, their well-documented adverse effects on the skin (Lachenmeier, 2022) create a clear need for safer, organic alternatives. Previous studies have demonstrated the antibacterial potential of eucalyptus-based ecoenzyme in other regions, such as Maluku (Rijal, 2021) however, the antibacterial effectiveness of eucalyptus leaf ecoenzyme produced by Lamongan MSMEs has not been investigated, which constitutes the novelty of this study. Accordingly, this research aims to determine the effectiveness of Lamongan-produced eucalyptus leaf ecoenzyme as a disinfectant, with the expected benefit of providing an environmentally friendly, low-side-effect alternative to chemical-based disinfectants and of adding scientific value to a locally abundant commodity.

This type of research is a comparative experimental study with a Random Group Design (RAK), which aims to test the effect of eucalyptus leaf ecoenzyme disinfectant with independent variables in the form of various ecoenzyme concentrations and bacterial growth in petri dishes as bound variables. The test was carried out by the finger swab method of people trying to Mueller Hinton Agar. The design of this study uses a comparison of each ecoenzyme concentration. The research sample consisted of 3 students of Universitas Airlangga who were selected using a simple random sampling technique, with 10 treatments on the fingers of each sample, namely the comparison of the concentration of eucalyptus leaf ecoenzyme liquid produced by Lamongan Regency MSMEs with aquades, namely 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, and 100% ecoenzyme concentration. The sample size of experimental research with a group random design can be simply formulated by Federer's formula as follows: $(t-1)(n-1) \geq 15$, where t is many groups/treatments and n is many samples per treatment.

RESEARCH METHODS

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Research design and site.

This study employed a comparative experimental design with a Randomized Group Design (RAK). The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Universitas Airlangga, from July 2023 to June 2024. The unit of analysis was the bacterial colony grown on culture media obtained from fingertip swabs of the research subjects. The independent variable was the concentration of eucalyptus leaf ecoenzyme (10%-100%), while the dependent variable was the number of bacterial colonies grown in the petri dishes.

Data source and collection technique.

The source of data was primary data obtained directly from three students of Universitas Airlangga who were selected through simple random sampling. Data were collected using the fingertip swab method, in which the swabs were inoculated onto Mueller-Hinton Agar after treatment with each ecoenzyme concentration. Ten treatments were applied to the fingers of each subject, corresponding to eucalyptus leaf ecoenzyme concentrations of 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, and 100% diluted with aquadest. The minimum sample size was determined using Federer's formula, $(t-1)(n-1) \geq 15$, where t is the number of treatment groups and n is the number of samples per treatment.

Data analysis.

The number of bacterial colonies was quantified as colony-forming units per square centimeter (CFU/cm2). The data were then analyzed using the Shapiro-Wilk test to assess the normality of the distribution, followed by a Paired T Test to determine whether there were significant differences in the number of bacterial colonies between ecoenzyme concentrations, with a significance level of 0.05.

RESULTS AND DISCUSSION

Results

In this chapter, the researcher explained the results of the use of eucalyptus leaf ecoenzymes produced by Lamongan MSMEs as a disinfectant in the form of *hand sanitizers*. This research was carried out in the microbiology laboratory of the Faculty of Medicine, Universitas Airlangga which started in July 2023 until June 2024.

Characteristics and Ecoenzyme Content of Eucalyptus Leaves

The manufacture of eucalyptus leaf ecoenzymes was carried out for 3 months (August 19, 2023 – November 11, 2023) with ingredients mixed in the form of 500 grams of brown sugar, 1500 grams of eucalyptus leaves, and 5000 ml of water (ratio of 1 brown sugar: 3 organic ingredients: 10 water). During the fermentation process, ecoenzymes are tightly closed and the lid is given a hose that leads to a bottle filled with water so that air from the outside cannot enter and the fermented gas does not accumulate in the tube.

The results of fermentation can only be taken after 3 months, in 3 months ecoenzymes there are changes in aroma and color. The aroma of ecoenzymes after 3 months becomes sour and light brown in color. After 6 months, ecoenzymes have their own characteristics, namely having a eucalyptus aroma that is not as strong as before and an increasingly sour smell. The color in the 6-month ecoenzyme also becomes browner. The consistency of ecoenzymes remains liquid from the beginning of manufacture until 6 months afterwards.

Table 1 Ecoenzyme characteristics test results at 0 months, 3 months, and 6 months

Aspects	Early ecoenzymes	Ecoenzyme 3 Months	Ecoenzyme 6 Months
Aroma	The eucalyptus aroma is intense and does not smell sour	The aroma of eucalyptus is still strong and smells sour	The aroma of eucalyptus is not as strong as before and it smells more and more sour
Color	Brown transparent	Light brown	Dark brown
Consistency	Liquid	Liquid	Liquid

Testing the content of acetic acid compounds was carried out using the HPLC (High Performance Liquid Chromatography) method at the Testing Service Unit of the Faculty of Pharmacy, Universitas Airlangga (ULPFFUA). The results of the content test on eucalyptus leaf ecoenzymes produced by Lamongan MSMEs found that there was no acetic acid content. Ecoenzyme acidity testing was carried out in the 6th month at the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga. The results showed that the eucalyptus ecoenzyme of Lamongan MSMEs was acidic with a pH of 4.95. In order to obtain truly sterile ecoenzymes, a sterilization test is first carried out at the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga so that it is known that the ecoenzymes to be used are free from microorgans. To minimize microorganism contamination after the sterilization test, the researchers conducted filtration on ecoenzymes and put them into sterile containers. The test results can be seen in the table below.

Table 2 Results of the ecoenzyme content test of eucalyptus Lamongan MSMEs

Test	Parameter	Method	Result
Kandungan	Asam asetat	HPLC	No Acetic Acid Contained in the Sample
Keasaman	Ph	pH meter	pH value = 4.95
Sterility	Microorganism	Inokulasi	No contaminant microorganisms were found on the test medium

Effect of Eucalyptus Leaf Ecoenzyme as a Disinfectant

Testing the effect of eucalyptus leaf ecoenzyme disinfectant was carried out by the swab method on the student's fingers. Ecoenzymes were diluted into 10 concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) and swabs were carried out using a sterile cotton swab on the student's fingers (1 finger gets 1 concentration). The swab results are put back into the concentration tube and stirred before swabbing on 1/4 of the plate. Each concentration is then intubated at a temperature of 37^{oC} for 1x24 hours.

3 sampling was carried out in this study in each treatment.Calculation of the number of bacterial colonies for each concentration is calculated from the number of bacterial colonies in the petri dish (*Colony Forming unit)* divided by 1/4 of the area of the petri dish (cm^2). The diameter of the petri dish in this study is 9 cm so that the area of the petri dish used is 16 cm^2 . The calculation results can be seen in table 5.1.3 below.

Table 3 Number of bacterial colonies in CFU/ cm2

Treatment	Iteration I	Repeat II	Repeat III	Average
100%	0.062	0.015	0	0.025
90%	0.042	0.007	0.003	0.017
80%	0.207	0.003	0.015	0.075
70%	0.023	0.046	0.019	0.029
60%	0.050	0.015	0.011	0.025
50%	0.074	0.023	0.007	0.034
40%	0.171	0.007	0.058	0.078
30%	0.589	0.011	0.093	0.231
20%	0.195	0.039	0.042	0.092
10%	0.121	0.082	0.031	0.078

At 10%, 20%, 30%, and 40% ecoenzyme concentrations, the number of colonies was higher than at 50%, 60%, 70%, 80%, 90%, and 100% concentrations. The 90% concentration has the least average number of bacterial colonies among other concentrations. Here is the average number of colonies, the number of colonies on samples one, two, and three on the line chart. In the diagram figure 5.1.1. There is no specific pattern of increase or decrease. In sample one, there was an increase from a concentration of 10% to 20% and reached a peak at a concentration of 30%, after which it gradually decreased to a concentration of 70% before rising again at a concentration of 80%, decreasing at a concentration of 90% and 100%. In sample two, the number of bacterial colonies initially decreased to a concentration of 40% before rising again at concentrations of 50%, 60%, and 70% and then falling back to concentrations of 80% to 100%. In the third sample, there was also an increase in the initial concentration of 10% to 30% before finally decreasing at 40% and 50% concentrations, then rising again to 80% concentrations, and finally decreasing at 90% and 100% concentrations.

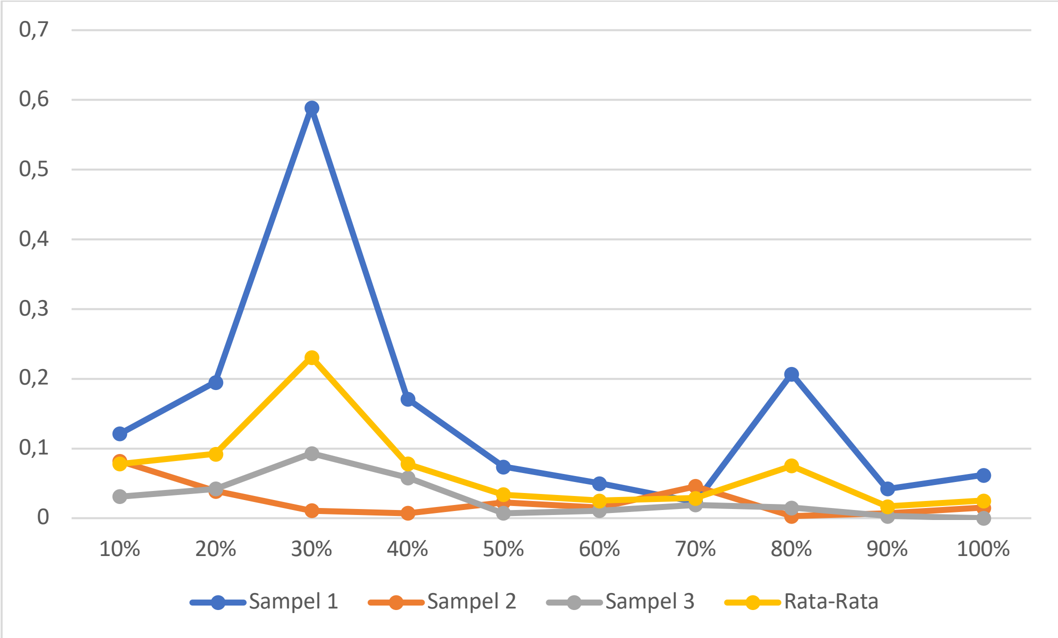

Figure 1 One, two, three, and average sample line diagrams

Table 4 Shapiro-Wilk Test Results

Tests of Normality

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	**df**	**Mr.**	**Statistic**	**df**	**Mr.**
100%	.296	3	.	.918	3	.447
90%	.352	3	.	.826	3	.178
80%	.367	3	.	.794	3	.100
70%	.335	3	.	.858	3	.263
60%	.352	3	.	.826	3	.178
50%	.297	3	.	.917	3	.441
40%	.264	3	.	.955	3	.590
30%	.337	3	.	.854	3	.251
20%	.341	3	.	.846	3	.230
10%	.202	3	.	.994	3	.853

Tabel 5 Results Paired T Test

Paired Samples Test

	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Number of Bacteria per treatment group 1 – Number of Bacteria per treatment group 2	0,055	0,163	0,047	-0,048	0,159	1.176	11	0,264

After calculating the number of colonies in each concentration, a normalitaas test was carried out using Shapiro Wilk and the results were insignificant which means that they were normally distributed (>0.05). Normal distribution is one of the requirements for the *Paired T Test*. Furthermore, in the *Paired T Test* , a significant result of 0.264 was obtained, which means that there was no significant difference in each experiment.

Discussion

Characteristics of Eucalyptus Leaf Ecoenzymes

Ecoenzymes are the result of fermentation of organic waste that is liquid, acidic and brownish in color (Dewi, Anugrah dan Nurfitri, 2017; Rijal et al., 2021). These characteristics show that the eucalyptus leaf ecoenzymes produced by Lamongan MSMEs have more or less the same characteristics as previous research. The harmonized ecoenzyme results show that the process of making eucalyptus leaf ecoenzymes has been carried out correctly. However, there is a difference in the results of content testing in eucalyptus leaf ecoenzymes produced by Lamongan MSMEs, namely the absence of acetic acid compounds. These results are not in line with previous studies that showed positive results (Rochyani, Utpalasari dan Dahliana, 2020; Rijal et al., 2021), so further analysis is needed to find out the cause of the difference in ecoenzyme content produced by Lamongan MSMEs in this study.

The difference in the raw materials used in this study with previous research conducted by (Rijal, 2021), can be the cause of the difference in content in eucalyptus leaf ecoenzymes produced by Lamongan MSMEs research uses sugar in the form of molasses (molasses), organic waste in the form of pineapples and papaya. Research by Rijal (2021) uses Molasses (molasses) as sugar and organic waste in the form of Maluku eucalyptus leaves and fruit waste (snakefruit, tomato, orange, and mango) (Rochyani, Utpalasari dan Dahliana, 2020; Rijal et al., 2021).

Differences in geographical location and environmental factors can also be the cause of the difference in content. The eucalyptus leaf samples taken in this study came from Lamongan which has an average rainfall of 50-150 mm per month. Maluku has a higher rainfall, which averages 200-400 mm per month (Central Statistics Agency of Maluku Province, 2022; Central Statistics Agency of East Java Province, 2018). Maluku has higher humidity than Lamongan which is more dominated by the dry season. The high humidity allows eucalyptus plants to produce more terpenoid volatile compounds such as sineol (Anwar, Pasaribu dan Nazari, 2022). Lower rainfall in Lamongan causes terpenoid compounds to be reduced to save resources when drought occurs (Anwar, Pasaribu dan Nazari, 2022).

The use of *eucalyptus leaves* as the main ingredient of ecoenzymes because it has terpenoid components that are anti-bacterial such as 1.8 sineol, α-pinene, β-pinene, and α-terpinolen (Bassolé dan Juliani, 2012; Hakim, Wilson dan Darmawati, 2019). These compounds work by damaging cell membranes, destroying genetic material, and inhibiting the work of bacterial enzymes (Carson, Mee dan Riley, 2002). The level of organic acids and terpenoids that can inhibit bacteria is proven by sterility tests. The results of the ecoenzyme sterility test of eucalyptus leaves produced by Lamongan MSMEs showed that there were no pathogenic bacteria in the inoculation results.

Effect of Eucalyptus Leaf Ecoenzyme Disinfectant

In this study, ecoenzymes did not show a significant decrease in the number of bacterial colonies after being given eucalyptus leaf ecoenzyme concentrations of 10%-100%. Eucalyptus leaf ekeonzyme contains terpenoids that are believed to be able to provide antibacterial effects (Rijal et al., 2021). The content of terpenoids that are able to kill bacteria is 12.4 µg/ml and to inhibit bacteria is 6.2 µg/ml (Wang et al., 2022). So it is possible that the eucalyptus leaf ecoenzymes produced by Lamongan MSMEs will not have a significant decrease in the number of bacterial colonies because the terpenoid content does not reach the minimum level to be able to inhibit or kill bacteria. In addition, the difference in the composition of the organic matter used also affects the diversity of compounds produced (Pebriani et al., 2022a). In the Rijal (2021) study, additional ingredients in the form of citrus fruits, salak fruits, mangoes, and tomatoes were used, while in this study only eucalyptus leaves were used. The difference in the composition of the material causes a difference in the content of the compounds produced, as evidenced by different pH levels.

Ecoenzymes are believed to be used as disinfectants because they contain organic acids that can inhibit the growth of pathogenic bacteria (W Negara, M Ridla, AD Lubis, W Winarsih, 2011; Welfalini, Suartha dan Sudipa, 2023). The organic acid content contained in fermentation products is acetic acid, lactic acid, citric acid and oxalic acid (Fajria et al., 2023; Ismail, Nainggolan dan Nauli, 2024). Based on research conducted by Rahmiati (2017), lactic acid has the ability to inhibit the growth of bacteria *Eschericia coli, Salmonella thypii, and Shigella dtsentriae* (Rahmiati, 2017). In the eucalyptus leaf ecoenzyme produced by Lamongan MSMEs, there is no level of acetic acid, which is one of the antibanteri ingredients (Baihaqi et al., 2024). The pH level of eucalyptus leaf ecoenzymes produced by Lamongan MSMEs also has a higher pH than the ecoenzymes of Andam and Rijal's research, which is around 4.95. The Andam and Rijal ecoenzymes have acidity levels of 3.81 and 4.2, respectively. The lower the acidity of the ecoenzyme, the better the quality of the ecoenzyme. This is because the more acidic the pH level of the ecoenzyme, the higher the organic acid content of the ecoenzyme (Larasati, Puji Astuti dan Triwahyuni Maharani, 2020). Organic acid levels can be increased when adding fruit base ingredients. Fruits will cause acid levels in the ecoonzyme to be higher. This condition allows the growth of lactic acid bacteria (BAL) that produce metabolic compounds in the form of lactic acid to grow properly. Therefore, lactic acid bacteria are found in large quantities in ecoenzymes that have additional ingredients from the fruit. Meanwhile, in this study, only one ingredient was used in the form of ecoenzyme leaves, so it was possible to have fewer lactic acid bacteria. This is evidenced by the highest level of acidity of eucalyptus leaf ecoenzymes produced by Lamongan MSMEs among other researchers, this means that the level of organic acids contained is lower. So that it can reduce the antibacterial effect of the ecoenzyme.

Rijal (2021) research stated that the ecoenzymes of Maluku eucalyptus leaves are able to inhibit the growth of bacteria *E. coli, S. aureus, dan C. albicans*. This research is contrary to the results of this study. One of the reasons is the difference in additional raw materials in ecoenzymes. Rijal (2021), in addition to using eucalyptus leaves as the main ingredient, also adds a mixture of fruits such as oranges, salak, mangoes, and tomatoes. This difference causes a difference in the diversity of compounds produced during the fermentation process (Pebriani et al., 2022).

Theoretical and Practical Implications

Theoretically, these findings extend the existing literature on plant-based ecoenzymes by demonstrating that the antibacterial efficacy reported for eucalyptus ecoenzyme in other regions (Rijal, 2021) is not automatically reproducible; rather, it is strongly conditioned by the raw-material composition, organic-acid content, and terpenoid concentration of the ecoenzyme rather than by the eucalyptus leaf substrate alone. The absence of acetic acid and the comparatively higher pH observed in the Lamongan-produced ecoenzyme support the theoretical premise that the antibacterial mechanism of ecoenzyme depends on reaching a minimum threshold of organic acids and terpenoids (Larasati et al. (n.d.). Compared with the study by Rijal (2021), which reported inhibition of E. coli, S. aureus, and C. albicans using multi-substrate ecoenzyme, the single-substrate formulation used in this study did not achieve a comparable effect, reinforcing the role of substrate diversity in fermentation outcomes (Pebriani et al., 2022b) Practically, these results imply that single-substrate eucalyptus ecoenzyme in its current formulation is not yet suitable as a standalone disinfectant, and that the addition of fruit-based organic materials to increase organic-acid content may be necessary to achieve a measurable antibacterial effect.

Research Limitations

During this study, there are several obstacles and complications that cause limitations in this study. The researcher hopes that with this evaluation, the next researcher can further improve the related research. The limitations of this study include that positive and negative controls are not used as pre and post comparisons, so it can only explain the presence or absence of disinfectant effects on each ecoenzyme concentration. Another limitation is that it cannot control the cleanliness of the hands of the sample of the trial person used. Although sampling is carried out randomly, it is undeniable that there are differences in activity in the test people before the bacteria are taken, allowing for a very large difference in the number of bacteria between the test people.

CONCLUSION

This study set out to determine the effectiveness of eucalyptus leaf ecoenzyme produced by Lamongan MSMEs as a disinfectant capable of replacing chemical-based disinfectants that have side effects on humans. In direct response to this objective, the study found the following. Based on the research on the effectiveness of eucalyptus leaf ecoenzymes produced by Lamongan MSMEs as disinfectants, it can be concluded that eucalyptus leaf ecoenzymes produced by Lamongan MSMEs do not have a significant disinfectant effect at any concentration of 10%-100%, and there is no significant difference in bacterial colonies in subjects given a concentration of 10%-100% ecoenzyme. Suggestions that researchers can propose for the progress of further research based on the obstacles and results of this study are as follows: first, conducting a lactic acid content test in ecoenzymes with the total BAL (Lactic Acid Bacteria) technique or using the HPLC (High Performance Liquid Chromatography) method to determine whether there is lactic acid content in the ecoenzymes produced by Lamongan MSMEs; second, conducting research trials using ecoenzymes that are less than 6 months old to compare the effectiveness of ecoenzymes in inhibiting bacteria; third, using the addition of other organic materials as a mixture of ecoenzymes, such as fruit and vegetable peels; and fourth, conducting a large test of the concentration of terpenoid active compounds contained in eucalyptus ecoenzymes by phytochemical or gas chromatography methods to ensure terpenoid levels that are able to inhibit bacteria.

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