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Antiseptic Gel Formulation Uses Eco-Enzyme from Rambutan (*Nephelium lappaceum*) Peels Substitute the Active Ingredient Alcohol

(Formulasi Gel Antiseptik Menggunakan Eco-Enzyme Dari Kulit Buah Rambutan (Nephelium lappaceum) Untuk Mensubstitusi Bahan Aktif Alkohol)

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ABSTRACT

Background: Eco-enzyme is the result of fermenting organic waste that possesses antibacterial properties. **Objectives:** This research aims to develop the best formula for an environmentally friendly antiseptic gel. **Methods:** The analysis carried out in this research includes testing the physical parameters and antibacterial activity of preparation. **Results:** The liquid eco-enzyme product produced has a distinct strong acid aroma with a pH of 3.1. Antiseptic gels were created in five formulations (F1-F5) by substituting the active alcohol ingredient traditionally used as an antibacterial agent. Test results showed that F1, F2, and F3 met all the requirements for the physical parameters of the gel, including pH (4.5-6.5), viscosity (2000-4000 Cp), spreadability (5-7 cm), and homogeneity (homogeneous). Meanwhile, F4 did not meet the physical parameter of viscosity, and F5 did not meet the parameters for viscosity and spreadability. Antiseptic gel made from eco-enzyme extracted from rambutan fruit peels exhibited higher antibacterial activity against S. aureus and E. coli bacteria compared to two commercial antiseptic gels and F1, which served as the negative control. **Conclusion:** An antiseptic gel preparation containing rambutan peel eco-enzyme with a concentration of 50% and 50% alcohol (F3) is the best formula.

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INTRODUCTION

Health is a crucial aspect of human life. Factors that can affect the quality of health include the presence of bacteria that can transmitted directly to humans. Bacteria that can easily transmitted through direct contact, namely *Staphylococcus aureus* and *Escherichia coli* bacteria often found on the palms of hands, are considered the primary pathogens for humans (Angga et al., 2015; Ramadhan, 2013). *Staphylococcus aureus* is a pathogenic bacterium that can cause various types of skin infections such as furuncles, carbuncles, and more serious infections like osteomyelitis, septic arthritis, and pneumonia. This bacterium can also cause illness through contamination of food and beverages by infected individuals' hands (Irianto, 2012). Additionally, transmission can occur through the air when someone coughs, sneezes, or spits.

Escherichia coli is another bacterium that can lead to infections through the skin, as the skin is a vulnerable organ for infections and other skin diseases (Oktalia, 2009). This bacterium can enter the human body via hands or contaminated objects such as bottles, pacifiers, thermometers, and eating utensils. Moreover, transmission can occur if someone touches the anal area and fails to wash their hands before touching nearby objects or consuming contaminated food. The use of antiseptics to maintain the cleanliness of hands, the body, and the environment is an essential step in preventing the contamination of these microorganisms.

Antiseptic is a substance that can inhibit or kill microorganisms by stopping their growth and metabolic activities (Asmawati and Setiawan, 2017). Commonly using synthetical chemical antiseptic agents can be replaced with more environmentally friendly natural substances. Fruit and vegetable waste can be utilized as natural ingredients to produce versatile antiseptics (Penmatsa et al., 2019). One of the fruit wastes is rambutan fruit peel. In 2023, the Central Sulawesi region reported a rambutan production of 11,036 tons, up from 9,872 tons in 2021, by the Central Bureau of Statistics (BPS). This abundant rambutan production also generates organic waste. Organic waste utilization derived from rambutan fruit peels can processed into eco-enzymes with various practical applications.

Eco-enzyme is a product resulting from the fermentation of organic waste composed of fruit and vegetable residues, brown sugar, and water in a ratio of 3:1:10, which lasts for 90 days (Larasati and Haribowo, 2020; Sathya Sai International Organization, 2021). Previous research conducted by Thalib (2022) regarding the testing of eco-enzymes derived from organic fruit and vegetable waste for use as a natural disinfectant found that eco-enzymes from rambutan fruit peel had the highest disinfection power with an average phenol coefficient value of 2.00. That indicates the eco-enzyme product is twice as effective in its antibacterial action more effective than phenol. Research also conducted by Marcy (2022) demonstrated that eco-enzymes from rambutan fruit peel exhibited the highest antimicrobial activity with an average inhibition zone diameter of 25.70 ± 0.40 mm against the test bacterium Salmonella typhi and 18.03 ± 0.91 mm against the test bacterium Staphylococcus aureus.

Research conducted by Wardhani and Supartono (2015) on the evaluation of the antibacterial activity of rambutan peel extract (Nephelium lappaceum L.) against *Escherichia coli* bacteria showed that the ethanol extract of rambutan peel has a higher antibacterial potential compared to the chloroform extract. The ethanol extract successfully inhibited the growth of *Escherichia coli* at a concentration of 40%, with an inhibition zone diameter of 6 mm.

The potential content of eco-enzymes from rambutan fruit peel offers an opportunity for its utilization as an antiseptic gel. Therefore, it is necessary to carry out the appropriate formulation in making hand antiseptic gel. The results of this research can provide a breakthrough by using the eco-enzymes from rambutan fruit peel as a natural alternative for active ingredients in antiseptic gel and support the development of environmentally friendly products.

MATERIAL AND METHODS

Materials

The type of research carried out is an empirical research approach with a laboratory experimental research platform. This research was conducted in 2023 for five months at the Microbiology Laboratory and Pharmaceutical Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University. Meanwhile, eco-enzyme and fungal particles separated in the Chemical Research Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University. The materials needed for making eco-enzyme include rambutan fruit as the research sample, palm sugar, and mineral water (Arifin et al., 2019). The materials used in the formulation of antiseptic gel include carbopol 940, 96% ethanol, glycerin, triethanolamine, and beauty water (Shu, 2013). Additionally, the test microbes (Staphylococcus aureus and *Escherichia coli*) and NA media used for evaluating the antibacterial activity of the antiseptic gel are included in this formulation (Dewi, 2010).

Methods

Preparation of Tools and Materials

The preparation of materials and equipment begins with gathering rambutan peel waste and the necessary ingredients, such as palm sugar, mineral water, and the supporting tools used during the research.

Preparation of Research Samples

The research sample consists of organic waste from rambutan fruit peels, often discarded in traditional markets, collected as much as 3 kilograms. Samples are washed clean and cut into small pieces so they are ready to be used for research.

Fermentation and Production of Eco-Enzyme (Arifin et al., 2019)

Organic waste 150 g, palm sugar 50 g, and 500 mL of water put into a fermentation vessel. Subsequently, it is fermented at room temperature for 90 days in a dry and sunlight-protected location. The resulting liquid eco-enzyme is then extracted by filtering and stored in a bottle for the subsequent formulation of an antiseptic gel.

Antiseptic Gel Formulation

The antiseptic gel formulation was made based on research conducted by Shu (2013). The formulation is prepared in five different formulas, each with varying compositions of alcohol and eco-enzyme, serving as an active antibacterial ingredient.

Table 1. Antiseptic Gel Preparation Formulation

Materials	Function	Total				
		F1	F2	F3	F4	F5
Alcohol 96% (mL)	Active ingredients	73.00	54.75	36.50	18.25	0
Eco-Enzyme (mL)	Active ingredients	0	18.25	36.5 0	54.75	73.00
Carbomer 940 (g)	Gelling agent	1	1	1	1	1
TEA (drops)	Alkalizing agent	2	2	2	2	2
Glycerin (mL)	Emollient	1.45	1.45	1.45	1.45	1.45
Beauty water (mL)	Solvent	Add 100	Add 100	Add 100	Add 100	Add 100

Observation and Data Analysis

The observations were physical parameters (pH, viscosity, spreading ability, and homogeneity) and antibacterial activity tests. The observation data obtained are analyzed using Analysis of Variance (ANOVA) and further tested using the Duncan test.

RESULTS AND DISCUSSION

Eco-Enzyme Product and Antiseptic Gel Formulation

The eco-enzyme product produced has a brownish color and a distinct strong acid aroma with a pH of 3.1. According to Win (2011), the fermentation of eco-enzyme can considered successful if it results in a solution with a brownish color, a distinct strong acid aroma, and a pH < 4, indicating an acidic pH.

Figure 1. Eco-enzyme products (a) and antiseptic gel preparations (b)

The antiseptic gel formulation made from rambutan fruit peel eco-enzyme shows that the higher the concentration of eco-enzyme added, the more intense the color and aroma of the resulting antiseptic gel formulation will become. The color will range from light brown to dark brown, and the aroma will also become strong.

Formulation of Eco-Enzyme Antiseptic Gel

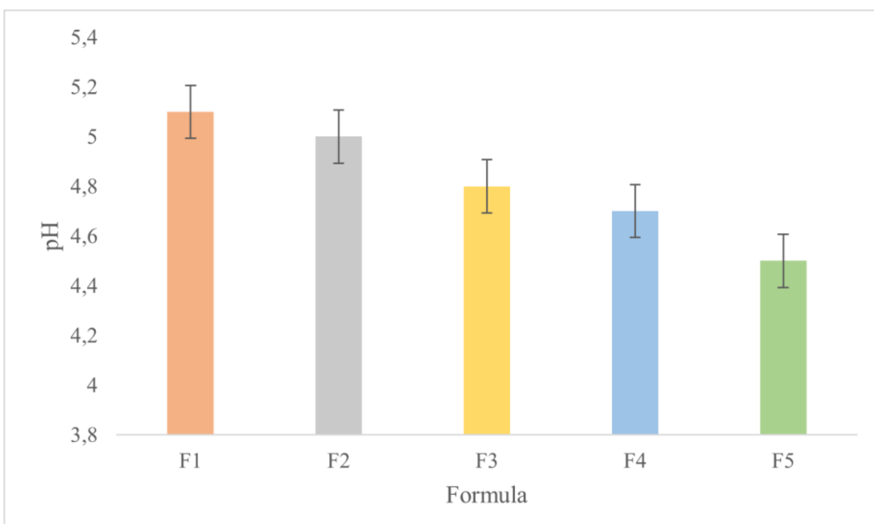

Figure 2. Results of pH measurements

Based on Figure 2, the pH measurements of the overall antiseptic gel formulations show varying values, with the pH decreasing as the amount of eco-enzyme increases. That is because the eco-enzyme from rambutan fruit peel has an acidic pH of 3.1. As the quantity of eco-enzymes from rambutan fruit peel increases, the pH becomes lower. The desired pH range for the gel is approximately 4.5-6.5, which aligns with the skin's pH conditions (Nikam, 2017). All formulations meet the required pH criteria.

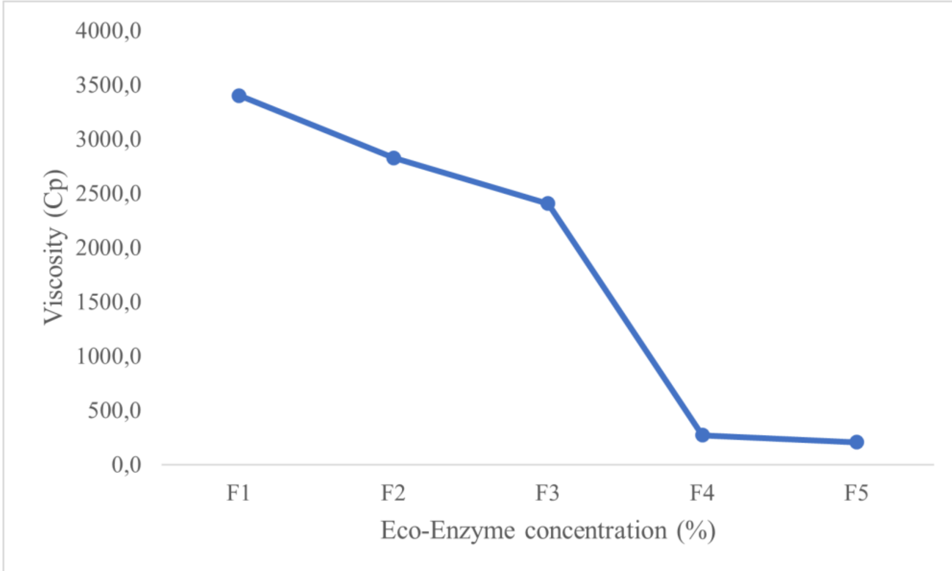

Figure 3. Results of measuring the viscosity of antiseptic gel preparations

The results of the Duncan test indicate that the concentration of F1 does not significantly differ from F2, the concentration of F2 shows a significant difference from F3, the concentration of F3 is not significantly different from F4, and the concentration of F4 significantly differs from the concentration of F5. Therefore, the concentration of eco-enzyme F2 provided in the antiseptic gel formulation does not have a significant impact on the viscosity of the antiseptic gel. However, at concentrations F3, F4, and F5, there is an effect on the viscosity of the antiseptic gel. These results suggest that the higher the concentration of eco-enzyme used, the lower the viscosity of the preparation. Figure 3 demonstrates the correlation between the increased concentration of eco-enzyme and the decrease in the viscosity curve. According to the research conducted by Noviardi et al (2018), using the same amount of carbopol and TEA in all formulations and increasing the amount of extract in the formula results in the formulation becoming more acidic, leading to a reduction in the number of ionized carboxyl groups. That, in turn, causes repulsion between carboxyl groups, resulting in a decrease in the development of the carbopol structure. That causes a decrease in gel viscosity as the extract amount increases.

The viscosity of a good antiseptic gel according to SNI 16-4399-1996 is 2,000–50,000 cP. Based on Figure 3, formulations F4 and F5 did not meet the expected viscosity range for antiseptic gels. This outcome was attributed to the high concentrations of eco-enzyme used, namely 75% and 100%, respectively. The use of carbopol and TEA in the same amounts as in formulations F1, F2, and F3, which contained eco-enzyme concentrations of 0%, 25%, and 50%, resulted in F4 and F5 exhibiting higher acidity. This condition reduced the number of ionized carboxyl groups, thereby decreasing the electrostatic repulsion between carboxyl groups. Consequently, the structural development of carbopol was not optimal, leading to a reduction in viscosity and causing formulations F4 and F5 to be more fluid compared to F1, F2, and F3.

Table 2. Results of Testing for Spreadability and Homogeneity of Antiseptic Gel Preparations

Parameter	Test Result				
	F1	F2	F3	F4	F5
Spreadability (cm)	5,8	6,1	6,1	6,4	9,3
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The required spreadability of the hand sanitizer should fall within the range of 5-7 cm (Yusuf et al., 2017). The test results for the spreadability of the formulations show that only F5 exceeds the required spreading power. This is due to F5 having a 100% concentration of eco-enzyme, where an increasing concentration of eco-enzyme results in a decrease in viscosity, thereby enhancing its spreadability. It is known that the eco-enzyme derived from rambutan peel has an acidic pH of 3.1 due to the formation of organic acids during fermentation, which decreases the ionization of carboxylate groups in carbopol and limits the expansion of the polymer network, thereby reducing gel viscosity. In addition, the presence of secondary metabolites, enzymes, and other bioactive compounds in the eco-enzyme may interact with polymers and pH adjusters, thus influencing the consistency and stability of the gel. Therefore, the acidity level and metabolite content of the eco-enzyme play a crucial role in regulating the balance between viscosity and spreadability in antiseptic gel formulations.

The clarity observed in the homogeneity assessment of all five gel formulations indicates an even distribution of all particles. This homogeneous distribution ensures that the active substance is uniformly spread in every application of the gel on the skin.

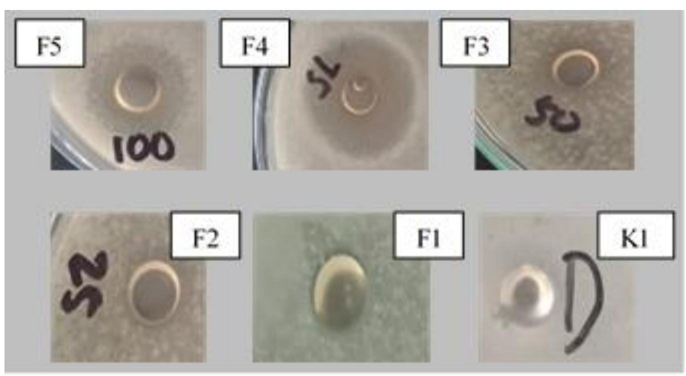

Figure 4. Results of antibacterial activity testing using the diffusion method against *S. aureus*

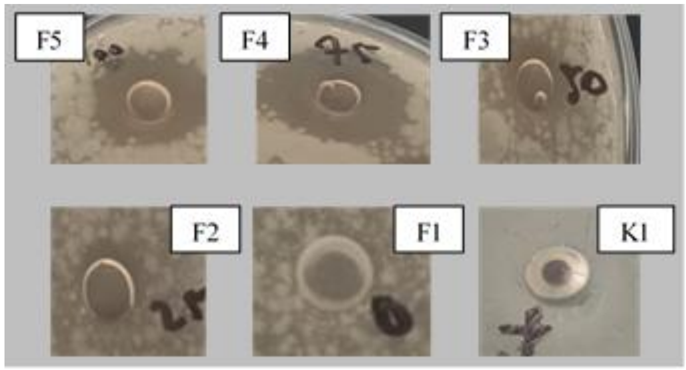

Figure 5. Results of antibacterial activity testing using the diffusion method against *E. coli*

Based on the results of further Duncan tests, each concentration of eco-enzyme exhibited significant differences. The test results indicated that the concentration of F2 significantly differed from F3, the concentration of F3 showed a significant difference from F5, and the concentration of F5 significantly differed from F4. That implies that the addition of eco-enzyme at concentrations ranging from 25% to 100% had a significant impact on the diameter of the inhibition zone produced. Thus, the data obtained demonstrate a significant effect on the antibacterial activity of the antiseptic gel formulation. The use of eco-enzyme from rambutan peel produces variations in antibacterial activity depending on its concentration. Based on the test results, higher concentrations of the active ingredient lead to larger inhibition zones. According to Arista's research (2013), there are four levels of antibacterial activity: very strong, strong, medium, and weak. If the diameter of the inhibition zone exceeds 20 mm, the formulation can be classified as having very strong inhibitory activity. Based on the results of the Duncan test, eco-enzyme produced the largest inhibition zone in formulations F4, measuring 20.64 ± 0.18 mm, and in F5, measuring 16.40 ± 0.15 mm against S. aureus bacteria. As for E. coli, formulation F3 exhibited an inhibition zone of 11.27 ± 0.42 mm, F4 showed an inhibition zone of 19.64±0.08 mm, and F5 had an inhibition zone of 17.36 ± 0.31 mm.

Table 3. Results of measuring the diameter of the inhibition zone for antiseptic gel preparations

Sample	Test bacteria *S. aureus*		Test bacteria *E. coli*	
	Diameter of the inhibition zone (mm)	Inhibitory power	Diameter of the inhibition zone (mm)	Inhibitory power
K 1	5,89 ± 0,03	Medium	6,42 ± 0,13	Medium
K 2	5,45 ± 0,09	Medium	6,28 ± 0,21	Medium
F1	5,70 ± 0,08	Medium	6,33 ± 0,06	Medium
F2	7,02 ± 0,02	Medium	7,62 ± 0,43	Medium
F3	7,79 ± 0,03	Medium	11,27 ± 0,42	Strong
F4	20,64 ± 0,18	Very strong	21,39 ± 0,08	Very strong
F5	16,40 ± 0,15	Strong	17,36 ± 0,31	Strong

Gel antiseptic with formulations F2 to F5 is highly effective in killing S. aureus and E. coli bacteria compared to two commercial antiseptic gel products with alcohol as the active ingredient (K) and F1, which serves as a negative control. That is evident from the larger inhibition zones of these formulations compared to the two commercial antiseptic gel products and F1 (Table 3). The antibacterial activity of the antiseptic gel using eco-enzyme from rambutan peel is likely due to the presence of organic acids and metabolite compounds with antibacterial properties. The fermentation process of eco-enzyme yields strong antimicrobial activity, capable of inhibiting microbial growth. This is attributed to the acetic acid (CH_3COOH) content in the eco-enzyme, which acts as an antibacterial agent (Rochyani et al., 2020). Research conducted by Wiyulyanto (2023) also indicates that the components of the eco-enzyme product from rambutan peel include acetic acid, methyl oleate, methyl palmitate, 1,2,3-benzenetriol, and sorbitol, all of which exhibit bioactivity in inhibiting or killing bacteria. The high phenolic content in eco-enzyme products made from organic waste, such as rambutan peel, may be influenced by secondary metabolites naturally present in the peel. According to Sulistyawati and Mulyati (2009), phenolic compounds act as antibacterial agents by disrupting the protein structure in cell membranes, leading to cell lysis and facilitating the penetration of phenolic compounds into the cytoplasm, ultimately inhibiting bacterial growth.

Samples F4 and F5 exhibit the largest inhibition zone diameters. However, their viscosity values exceed the required viscosity of gel formulations. Based on the results of physical parameter testing and antibacterial activity, formula F3 stands out as the best formulation for an antiseptic gel.

CONCLUSION

Based on this research, it can be concluded that the eco-enzyme extracted from rambutan fruit peels exhibits characteristics of a brownish color, and a distinct strong acidic aroma (pH 3.1). The best formulation for an eco-enzyme antiseptic gel is a preparation containing a 50% concentration of eco-enzyme from rambutan fruit peels and 50% alcohol (F3), meeting the requirements for an antiseptic gel formulation. This gel also demonstrates higher antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* compared to two commercial antiseptic gels available in the market, with inhibition zones measuring 9.35 ± 0.43 mm and 11.27 ± 0.42 mm, respectively. Therefore, eco-enzymes from rambutan fruit peels hold potential as a primary ingredient for the development of effective antiseptic products that can compete with existing commercial products.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest with the research, writing, and/or publication of this article.

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