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The effectiveness of salak pondoh peel ecoenzyme spray gel (*Salacca edulis* Reinw.) in pre-clinical evaluation of grade II burn healing

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ABSTRACT

Burns are a serious health problem and require proper treatment for healing. Salak pondoh (*Salacca edulis* Reinw.) peel is known to contain high flavonoid compounds that have potential as antioxidants and antimicrobials. This study aims to determine the antibacterial activity of salak pondoh peel ecoenzyme and evaluate the effectiveness of its spray gel in healing burn wounds in rats. The research began with the preparation of eco-enzyme, flavonoid test using tube test and spectrophotometry, antibacterial test using disc diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, formulation and spray gel test, antioxidant test using DPPH method, and in vivo test to observe the percentage of second degree burn wound healing in male Wistar rats. The *in vivo* experiment involved 28 rats divided into five treatment groups: Control (K), Positive Control (KP), Negative Control (KN), 15% spray gel (P1), and 20% spray gel (P2). Data from *in vitro* and *in vivo* tests were analyzed using One-Way ANOVA (Analysis of Variance) with a significance level >0.05, followed by Tukey's Posthoc test. The results showed that 15% salak pondoh peel eco-enzyme showed strong antibacterial activity with an inhibition zone of 11.83 ± 0.76 against the growth of *S. aureus* and 10.83 ± 0.28 in the inhibition zone of *P. aeruginosa*. The 20% concentration (P2) showed the highest effectiveness in accelerating burn wound healing in test rats, with a healing rate of 99% ($p < 0.05$) on day 18. In conclusion, salak pondoh peel eco-enzyme showed antibacterial activity, and the eco-enzyme spray gel effectively accelerated burn wound healing.

Keywords: Burns, ecoenzyme, salak pondoh peel, spray gel

INTRODUCTION

In Indonesia, the death rate from injuries is still high at around 75%, mainly due to burns (Eka et al., 2021). Data from the Ministry of Riset Kesehatan dasar (Riskesdas) in 2018 states that the prevalence of burns increased from 0.6% to 1.3% in the Indonesian population. The Basic Health survey report noted that there are more than 40% of patients experiencing second-degree burns with a mortality rate of 37%-39% per year. Therefore, further handling is needed to reduce this figure. One potential solution is the use of natural ingredients as an alternative to burn treatment, such as ecoenzymes.

Ecoenzyme is a fermentation product of organic waste with the addition of sugar and water as supporting ingredients. This fermentation process produces bioactive compounds such as gas, alcohol, organic acids (including acetic acid and lactic acid). Ecoenzymes are acidic due to the formation of organic acids from carbohydrates. Glucose is converted into pyruvic acid, which under anaerobic conditions is converted into ethanol and carbon dioxide. Ethanol is then oxidized by Acetobacter bacteria into acetic acid. After complete fermentation, eco enzymes are formed that can be utilized as antimicrobial, antioxidant, and environmental cleaning activities (Zaki et al., 2024). Ecoenzymes are not only beneficial for industrial and household applications, but also have the potential to be bioactive ingredients for medical purposes, such as healing wounds caused by sharp objects, cuts, scratches, ulcers, relieving skin infections, and treating burns (Riyanti et al., 2023).

Salak pondoh peel (*Salacca edulis* Reinw.) is known to have antioxidant activity with an IC50 value of 99.1 μg/mL (Rusdi, 2024). In addition, salak pondoh peel also contains flavonoid compounds with a total flavonoid level of 0.295 ± 0.00033 μgQE/μl which has the potential to prevent oxidation and inhibit toxic substances that can arise in wounds. Salak pondoh peel contains a number of bioactive compounds, including flavonoids, saponins, tannins, and polyphenol, each of which acts through different mechanisms as both antibacterial and antioxidant. Flavonoid compounds have a mechanism as antibacterial by forming complex compounds with extracellular proteins that can damage the bacterial cell membrane and then the release of intracellular compounds so as to inhibit the function and metabolism of cell wall membranes and. Flavonoids also have the ability to neutralize free radicals, thereby reducing oxidative stress in wound tissue and accelerating the healing process (Lubis., J. A., et al, 2024; Saptowo & Supriningrum, 2022). Saponins can increase fibroblast migration and proliferation, and tannins have antibacterial activity and increase angiogenesis. Polyphenols has anti-inflammatory, antibacterial and antioxidant properties and specifically improves wound healing (Safira & Ratih, 2023). These compounds can also help reduce the risk of infection in open wounds and speed up the healing process. Salak pondoh peel can be processed into ecoenzymes (Robiyyan et al., 2021). Some research results show that ecoenzymes have antibacterial activity against several pathogenic bacteria such as *Staphylococcus aureus* bacteria and *Pseudomonas aeruginosa* (Amalina et al., 2022).

Previous research has stated that salak pondoh peel ecoenzymes can be processed into a gel to heal burns in rats, with results comparable to commercial burn gel products (Mutmainah et al., 2017). To increase the efficacy of use, salak pondoh peel ecoenzyme can be developed in the form of a spray gel preparation. Spray gel preparations have several advantages, such as lower levels of microorganism contamination, longer drug contact times, and ease of use (Rizal et al., 2023). This discovery strengthens the potential of salak pondoh peel ecoenzymes as the main ingredient of topical preparations for burn healing. However, research on ecoenzymes from salak pondoh peel (*Salacca edulis* Reinw.) is still limited, especially at the pre-clinical stage (both *in vitro* and *in vivo*). Therefore, further development is needed to evaluate the antibacterial activity of salak pondoh peel ecoenzyme in inhibiting the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as to determine the effectiveness of ecoenzyme spray gel in healing burns in rats.

METHOD

Ethical clearance

This research procedure has been approved by the Ethics Committee with the code (No. 012405092) of Ahmad Dahlan University to ensure that the research carried out is in accordance with the principles of the research.

Ecoenzyme manufacturing and pH and total acetic acid tests

The main raw material for ecoenzymes is the peel of salak pondoh fruit (*Salacca edulis* Reinw.) which is taken from plantations in the Kembangarum area, Turi on Yogyakarta. The production of ecoenzyme is carried out by taking 800 grams of Pondoh salak peel, which has been thoroughly washed, adding 200 grams of molasses, and 2000 mL of water (with a ratio of 3:1:10), and mixing them in a jar until homogeneous. Fermentation is performed for 1 month and stored in a closed container. Afterward, filtration is done to obtain the pure eco-enzyme solution, followed by pH determination and total titratable acid tests.

The pH testing of the solution was carried out following the method of Azizah et al. (2022), namely using a pH meter. The measurement of total ecoenzyme-titrated acid was carried out through a quantitative test using alkalimetry titration with NaOH as the titer. The NaOH standard used was with a concentration of 0.1 N. The testing procedure was carried out according to Audy (2022), namely by taking as much as 10 mL of eco-enzyme then given 3 drops of the 1% phenolphthalein indicator (PP), then titrated with NaOH 0.1 N until it changed color and the volume used was recorded. Total titrated acid is calculated as acetic acid, with the following calculation formula:

$$\frac{\boldsymbol{M\ NaOH} \times \boldsymbol{V\ NaOH} \times \boldsymbol{Mr}}{\boldsymbol{V\ Sampel} \times \mathbf{1000}} \times \mathbf{100\%}$$

Description :

M NaOH = Molarity of the NaOH used (N)
V NaOH = Volume of NaOH used for titration (mL)
Mr = Relative molecular mass of the compound
Vol sampel= Volume of eco-enzyme used (Ml)

Flavonoid test

The flavonoid test was carried out by taking 1 ml of ecoenzyme solution and putting it into a test tube, then adding methanol as much as 1.5 ml, 0.1 ml of AlCL3, 0.1 ml of CH3COOK, and 2.8 ml of aquades, then divortex for 10 seconds. Discoloration is observed. Analysis of total flavonoid levels was measured on a UV-Vis spectrophotometer with a wavelength of 440 nm (Kusnadi & Devi, 2017).

Total flavonoid content

$$\text{(mg QE/g sample)}= \frac{C \times V}{m}$$

Description :

C = Flavonoid concentration obtained from the calibration curve (mg/mL)
V = Total sample volume (mL)
M = Sample massa (g)

In vitro assay (antibacterial test)

In vitro testing (antibacterial activity test) is carried out by the disc diffusion method. The positive control used chloramphenicol as much as 15 mg (in 5 ml aquades) and the negative control used sterile aquades. The disc paper is soaked in Salak Pondoh peel ecoenzyme solution for 15 minutes so that the ecoenzyme solution absorbs perfectly. Furthermore, the disc paper was placed on the media that had been planted with *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria in 10%, 15%, 20% treatment respectively and triplo repeats were carried out. Then the clear zone growing on the surface of the medium is observed and measured in diameter.

Spray gel manufacturing and testing

The preparation of salak pondoh peel ecoenzyme spray gel uses as shown in Table 1. First, the carbuncle and HPMC are mixed in a beaker glass, then 8 drops of TEA are added to the carbopol until it forms a gel mass, then HPMC is added to grind until homogeneous. Mix propylene glycol, methyl paraben, propyl paraben, ethanol and aquades stirred until homogeneous. Ecoenzyme solutions are inserted according to the formulation, namely 15 and 20 ml, then put into a spray bottle (Azizah, 2024).

Table 1. Formulation for the preparation of salak pondoh peel eco-enzyme spray gel.

Formula components	Composition	
	F1 (15%)	**F2 (20%)**
Ecoenzyme of salak pondoh peel (ml)	15	20
Karbopol (gr)	0,5	0,5
HPMC (gr)	0,5	0,5
Trietanolamine (gr)	8	8
Propilen Glikol (gr)	15	15
Metil Paraben (gr)	0,18	0,18
Propil Paraben (gr)	0,2	0,2
Etanol (ml)	20	20
Aquadest (ml)	Add 100	Add 100

(Source: Azizah, 2024)

Physical evaluation includes organoleptic tests, dispersibility, spray conditions, and inherent resistance properties. Organoleptic tests are carried out to see the physical appearance of the preparation by observing the shape, color, and smell of the preparation that has been made. The homogeneity test was carried out by applying the preparation to the glass preparation, then flattened by attaching the glass preparation and observed. Observations were seen in the absence of particles or evenly dispersed. Next, a pH test was carried out using a pH meter that had been calibrated by placing as much as 50 mL of the preparation into a beaker glass and then the electrode was dipped in the preparation. The dispersion test was carried out by spraying the preparation on mica plastic with a distance of 5 cm. Then the dispersion of the preparation was measured using a ruler. The stickiness test is carried out by spraying spray gel at a distance of 3 cm. Spray gel droplets that drip after 10 seconds are evaluated as dripping, and when spray gel droplets do not drip after 10 seconds, they are evaluated as attached (Fitriansyah et al., 2016).

Antioxidant test

The antioxidant activity test was carried out using the DPPH method. The sample was weighed 1.5 grams, then dissolved using methanol at a certain concentration. 1 ml of the parent solution is taken and inserted into the test tube, 1 mL, 200 μM DPPH solution is added. Incubate in a dark room for 30 minutes. The solution is diluted to 5 ml using methanol. The blank solution is made with 1 ml of DPPH solution plus 4 ml of ethanol. The absorbance value is measured at a wavelength of 515 Nm.

In vivo tests

The in vivo test involved the treatment and observation of rats subjected to burn wounds and the measurement of burn wound healing diameter. A total of 28 male Wistar rats were used. This study used a completely randomized design (CRD). Randomization was carried out into 5 treatment groups, namely K (no burns and spray gel), KN (burns, no spray gel), KP (burns and spray medicine (Pure Kids Wound Care Antiseptic), P1 (burns and spray gel 15%), and P2 (burns and spray gel 20%). The test animals underwent a 7-day acclimatization period. The procedure continued with the infliction of burn wounds and the application of spray gel. The rats' fur was shaved in the back area, covering 4 cm^2, and cleaned with cotton soaked in 70% alcohol. A stainless steel plate with a 2 cm diameter was heated to 40-50°C and applied to the rats' backs for 10 seconds until blistering occurred (Hendi and Nyoman, 2019). The application of spray medicine and eco-enzyme spray gel was carried out for 18 days. Observations were based on the percentage of burn wound healing (Paul et al., 2021) as follows:

$$\text{Percentage of recovery} = \frac{D1 - MDx}{D1} X\ 100\%$$

Description:
D1 = Diameter of the wound on the first day.
MDx = Mean (average) wound diameter on the observation day.

Data analysis

The data on econzyme characteristics in the form of pH, total acetic acid, and total flavonoids were analyzed descriptively quantitatively. In vitro data were analyzed using One Way ANNOVA (*Analysis of Variance*) with a significance level >0.05, if there was a significant difference then continued with Tukey's Posthoc test. While in vivo data were analyzed using Independent Samples T-Test (sign value <0.05).

RESULTS AND DISCUSSION

Burns can trigger high oxidative stress due to increased free radical production, which if not controlled will inhibit the tissue healing process. One natural alternative that could potentially be used to treat this condition is ecoenzyme. Ecoenzyme is a liquid fermented from organic waste such as fruit and vegetable peels that is rich in bioactive compounds. The main ingredients in ecoenzyme include flavonoids, polyphenols, vitamins C and E, proteolytic enzymes, and various important minerals. These compounds are known to have strong antioxidant, antibacterial and anti-inflammatory activities (Robiyyan et al., 2021).

Table 2. Characteristics of salak pondoh peel ecoenzymes

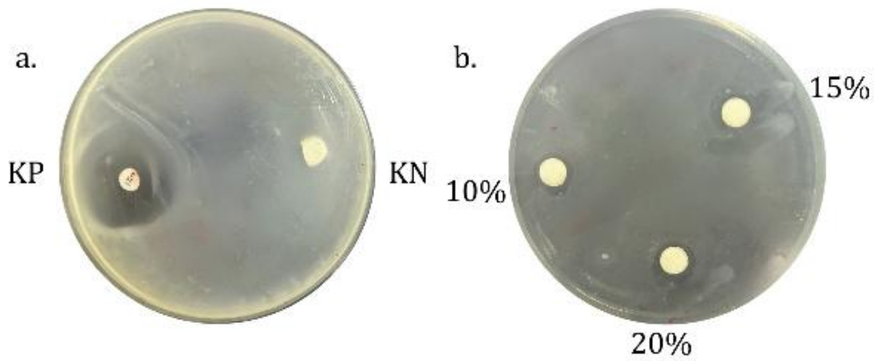
No	Characteristic test	Results
1.	pH	3,99
2.	Total acetic acid (%)	2,26 ± 0,12
3.	Total Flavonoid levels (mgQE/g)	4,01

Based on the results of the ecoenzyme characteristic test, a pH value of 3.99 was obtained, which indicates a weak acidic condition. This acidic pH is important because it can create an environment that does not support the growth of pathogenic microorganisms, thus helping to prevent secondary infections in burns (Gumilar et al., 2023). In addition, the relatively low pH is still considered safe for topical application with minimal risk of irritation, which is close to normal skin pH (4.5-5.5) (Gesper & Herlina, 2022). The total acetic acid contained in ecoenzyme of 2.26% ± 0.12 plays an important role as a natural antimicrobial agent and helps maintain the pH stability of ecoenzyme (Audy, 2022). Acetic acid is known to inhibit the growth of pathogenic bacteria, thus supporting the wound healing process by preventing infections that can slow down tissue regeneration (Schäfer & Werner, 2018). In addition, the total flavonoid content of the ecoenzyme of 4.01 mgQE/g provides significant antioxidant and anti-inflammatory effects. Flavonoids help neutralize free radicals generated due to oxidative stress in burns, reduce inflammation, and stimulate collagen formation and epithelialization, which are important for skin tissue repair (Robbiyan et al., 2021). The synergy between acidic pH, acetic acid content, and flavonoids in ecoenzymes provides a multifaceted effect that supports optimal burn wound healing. With an appropriate pH, ecoenzyme not only protects the wound from infection but also increases the body's endogenous antioxidant activity. The significant flavonoids and acetic acid content strengthen the potential of ecoenzyme as an effective, safe, and environmentally friendly alternative to natural topical therapy (Susilowati & Estiningrum, 2016).

Table 3. Results of the ecoenzyme antibacterial test

Treatment	Bacteria *S. aureus*		Bacteria *P. aeruginosa*	
	Inhibition Zone (mm)	Activity	InhibitionZone (mm)	Activity
Negative Control	0 ± 0	weak	0 ± 0	weak
Positive Control	11.83 ± 10.25^{c}	strong	7,83 ± 0,28^{b}	medium
Concentration 10%	10.16 ± 0.76^{b}	strong	9,33 ± 0,28^{c}	medium
Concentration 15%	11.83 ± 0.76^{c}	strong	10,83 ± 0,28^{d}	medium
Concentration 20%	11.00 ± 0,86^{bc}	strong	8,33 ± 0,28^{e}	medium

Description (Nisa et al., 2024) :
<5 mm = Weak activity
5-10 mm = Medium activity
>10-20 mm = Strong Activity
> 20-30 mm = Very Strong Activity



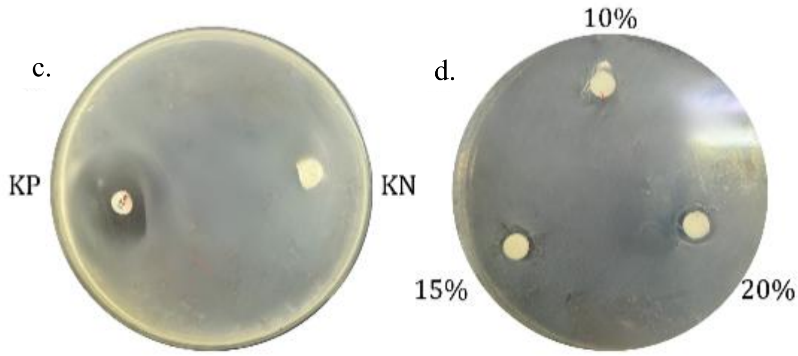


Figure 1. Diameter Diameter of the inhibition zone: (a) Control *S. aureus* ; (b) Treatment *S. aureus*, (c) Control *P. aeruginosa*, (d) Treatment *P.* aeruginosa. Description: Positive Control (KP), Negative Control (KN).

The test results of table 3 showed that the positive control (11.83 ± 10.25 mm for *S. aureus*; 7.83 ± 0.28 mm for *P. aeruginosa*) and 15% concentration treatment (11.83 ± 0.76 mm for *S. aureus*; 10.83 ± 0.28 mm for *P. aeruginosa*), in which 15% salak pondoh peel eco-enzyme showed strong antibacterial activity and did not show a significant difference with the positive control. However, both treatments showed a statistically significant difference compared to the negative control, which did not produce an inhibition zone (0 ± 0 mm for both bacterial strains). The results of the analysis showed that the antibacterial activity was distributed normally and homogeneously with a sign value of > 0.05, so it was further tested with *the One Way ANNOVA (Analysis of Variant*) test which showed a sign value. >0.05 at a 95% confidence level. The follow-up test used the *Tukey Posthoc Test* that p>0.05 there was no significant difference between the two groups.

Figure 1 shows that the negative control using distilled water exhibited no antibacterial activity, whereas the positive control using chloramphenicol demonstrated strong antibacterial activity against *Staphylococcus aureus*. The negative control made of sterile distilled water showed no activity and a significant difference compared to the positive control. The antibacterial activity of the eco-enzyme against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a 15% concentration was classified as strong and exhibited greater inhibition compared to other concentrations. Eco-enzymes containing acetic acid, alcohol, and low pH serve as effective natural antibacterial alternatives. Additionally, eco-enzymes contain active flavonoid compounds that can inhibit bacterial growth by damaging the cell walls (Febriani et al., 2024). Wardani (2020) stated that flavonoids play an important role in the wound-healing process by inhibiting bacterial growth. Their mechanism includes damaging bacterial cell walls, disrupting the cytoplasmic membrane, and inhibiting bacterial mobility (Wardani, 2020).

Nisa et al. (2024) stated that Gram-negative bacteria *P. aeruginosa* exhibit stronger resistance compared to Gram-positive bacteria *S. aureus* due to their cell wall structure. Gram-positive bacteria have a thick peptidoglycan wall, making them more tolerant to high osmotic pressure. In contrast, Gram-negative bacteria possess three cell envelope layers, consisting of a thin peptidoglycan layer sandwiched between an outer membrane and an inner membrane, providing additional protection (Amalina et al., 2022). Microorganisms in eco-enzymes produce antimicrobial compounds such as flavonoids, which function by damaging the bacterial cell membrane structure. The positive charge on the antibacterial agent is attracted to the negative charge on the bacterial cell membrane, causing instability and damage to the membrane. Additionally, these antibacterial agents can interact with peptidoglycan, the main component of bacterial cell walls, particularly in Gram-positive bacteria. This interaction disrupts the integrity of the cell wall, leading to bacterial cell death (Tavares et al., 2020).

Table 4. Ecoenzyme gel spray test results.

Evaluation	P1 (15%)	P2 (20%)
Smell	Slightly pungent	Pungent
Color	Light Brown	Dark Brown
Homogenity	Homogeneous	Homogeneous
pH	4.82	4.71
Number of spray gels within a spray distance of 5 cm	87.80 mL	83.10 mL
10 seconds stickiness test	Non-sticky	Non-sticky

Table 4. Two ecoenzyme spray gel formulations, P1 (15%) and P2 (20%), showed relatively stable physical characteristics and were organoleptically and functionally acceptable. In terms of odor, P1 has a slightly pungent aroma, while P2 (20%) shows a more pungent odor, which can be attributed to the high concentration of ecoenzyme and the accumulation of volatile compounds, such as acetic acid and other fermentation components (Widiani, 2023). The color of P1 (15%) tended to be lighter (light brown), while P2 (20%) appeared more intense (dark brown), in line with the higher concentration of ecoenzyme active ingredients. This also shows the visual stability of the formulation without any precipitation or separate phases, which is reinforced by the homogeneity test results which state that both preparations are homogeneous (Rokhmah et al., 2021). The pH values of each formulation were 4.82 (P1) and 4.71 (P2), still within the safe range for topical application (around 4.5-6.0) and in accordance with the pH of normal skin. This slightly acidic pH supports an antibacterial environment that is inhospitable to pathogens, while being gentle to wound tissue, so that it can accelerate wound healing without causing irritation (Wijaya et al., 2024). The amount of spray gel released at a spraying distance of 5 cm was 87.80 mL for P1 and 83.10 mL for P2. This difference indicates that the possibly higher viscosity or specific gravity of P2 slightly affects the spray volume. However, this value is still within the practically acceptable range for topical spray use. The stickiness test showed that both formulations were non-sticky for 10 seconds after application. This is an important advantage as non-sticky spray gels are more convenient to use, especially for burn patients who experience high sensitivity of the injured skin. Overall, the P1 and P2 ecoenzyme spray gel formulations exhibited good physical stability, appropriate pH, and optimal comfort of use. These formulations also retained the bioactive properties of the ecoenzyme, such as antioxidant and antimicrobial activities, derived from the flavonoids and acetic acid content (Hayati et al., 2019).

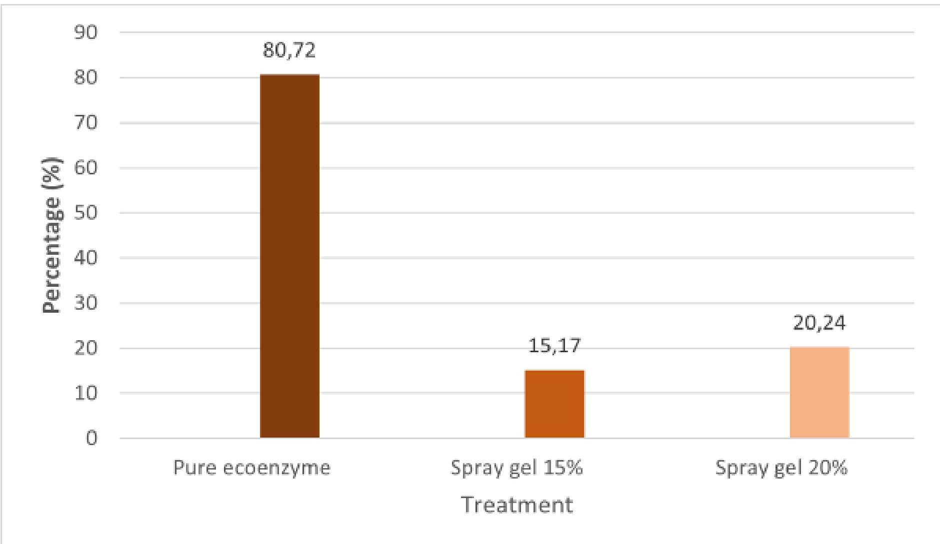


Figure 2. Antioxidant test result diagram

Figure 2 showed that the antioxidant activity of pure ecoenzyme reached 80.72%, while the antioxidant activity of spray gel with 15% and 20% concentration was 15.17% to 20.24%. The highest antioxidant effectiveness was seen in the use of pure ecoenzyme, which indicates that the active compounds in it such as acetic acid and flavonoids work best without mixing other ingredients. However, the decrease in effectiveness in the 15% and 20% spray gel formulations is likely due to the dilution of the ecoenzyme in the gel base, which may reduce the concentration of bioactive compounds. Nevertheless, spray gel still has advantages in terms of topical application which is more practical and convenient. This makes spray gel a potential alternative application, especially in the context of topical therapy for mild to moderate wounds. And the 20% concentration spray gel formulation has a higher effectiveness than 15%, indicating a positive relationship between increasing the concentration of ecoenzymes in the formulation and product effectiveness. According to Pratiwi et al. (2023), the high antioxidant activity of the ecoenzyme spray gel is influenced by the high flavonoid content, acetic acid content, and pH of the formulation which is in the mild acid range (4.71-4.82), which supports the stability of antioxidant compounds. Increasing the concentration of ecoenzyme in the spray gel also contributes to the increased antioxidant effectiveness, as it enriches the content of bioactive compounds such as flavonoids and organic acids. The combination of these factors makes the ecoenzyme spray gel an alternative topical preparation that has the potential to support the wound healing process through antioxidant mechanisms (Mardiyati & Sutiningsih, 2019).

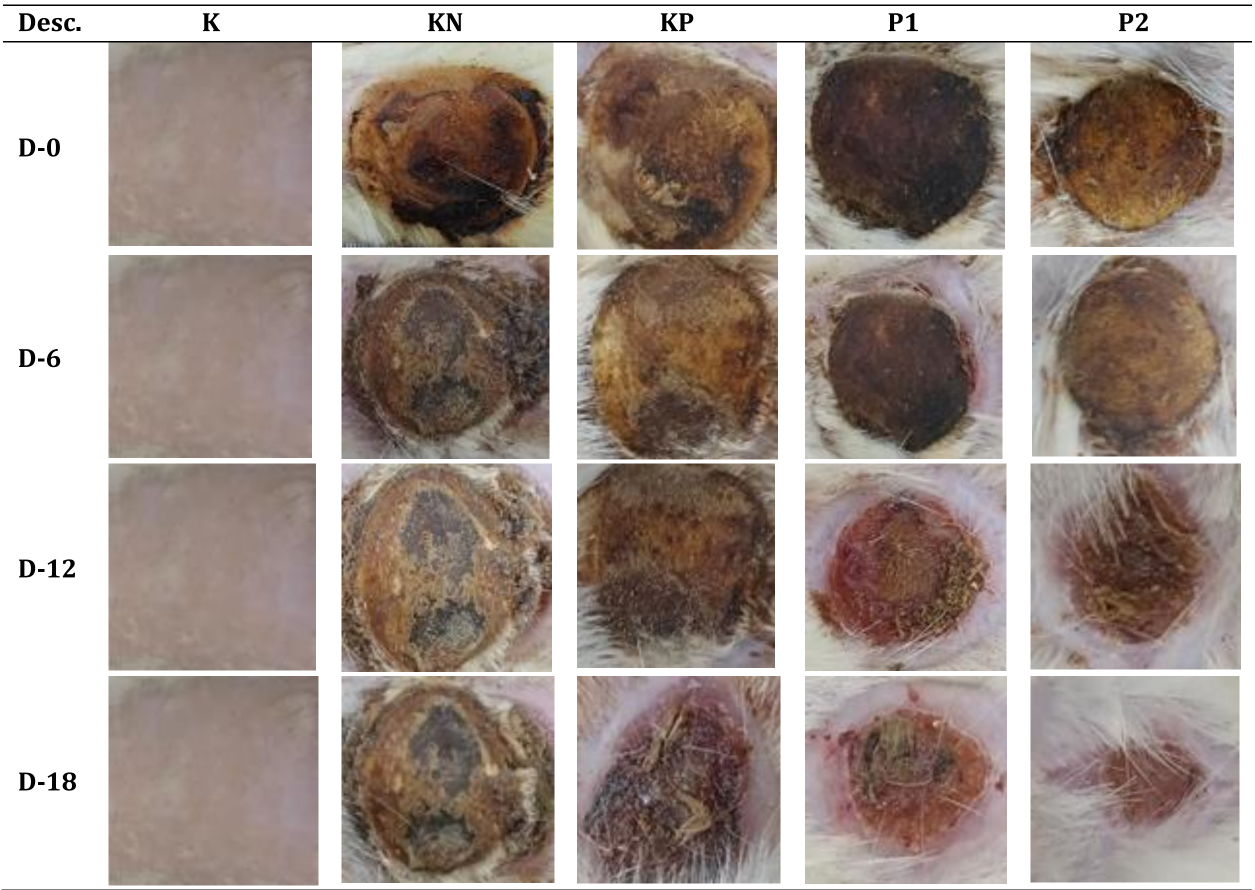
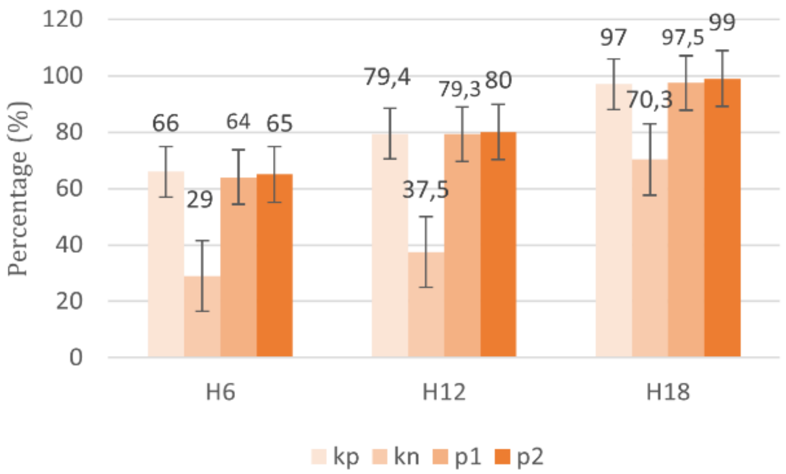


Figure 3. Observation results of burns with a diameter of 2x2 cm.

Figure 3 shows that on day 18 of observation, P2 (20%) demonstrated the best results compared to other treatments. The burn wound condition in P2 (20%) was almost completely closed, with the wound area significantly reduced and new hair growth observed. According to Rokhmah et al. (2021), burn wound healing requires time, resulting in a longer healing process for the negative control group, positive control group, and P1 (15%). By the end of the study on day 18, none of the test animals in these groups showed complete skin closure improvement.



Description : Control positive (kp), Control negative (kn), p1 (15%), p2 (20%). Mice were acclimatized for 7 days. Observations were made on Day (D-6, D-12, D-18).

Figure 4. Burn wound healing percentage diagram.

Figure 4 shows that the ecoenzyme gel spray from salak pondoh peel, especially at a concentration of 20% (P2), showed the highest effectiveness in accelerating the healing of burn wounds in test rats, with the percentage of healing reaching 99% on day 18. This result was slightly higher than the positive control (97%) and P1 (97.5%), and significantly higher than the negative control (70.3%).

The results of the analysis showed that the data on the percentage of wound healing between group P2 (20%) and the positive control were normally distributed and homogeneous (significance > 0.05), so it was continued with the Independent Samples T-Test test. The significance value <0.05 indicates that there is a statistically significant difference between the two groups. Thus, it can be concluded that the P2 (20%) concentration of salak pondoh peel ecoenzyme spray gel provides a comparable or even better burn wound healing effect compared to the positive control. This indicates that ecoenzyme has the potential as an alternative topical agent in burn therapy.

Ecoenzyme spray gel has shown effectiveness in supporting the burn wound healing process. The healing mechanism itself has three main phases, namely inflammation, proliferation, and remodeling (Asri, 2017). In the inflammatory phase, flavonoids in ecoenzyme act as antioxidants that are able to neutralize excess free radicals (ROS) produced during the inflammatory process, thus preventing further cell damage and reducing the inflammatory response. Flavonoids are also known to suppress proinflammatory cytokines such as TNF-α and IL-6. In addition, the antibacterial activity of acetic acid in ecoenzymes helps prevent secondary infections that can worsen inflammation. In the proliferation phase, flavonoids and phenolic compounds stimulate fibroblast proliferation, accelerate epithelialization, and support angiogenesis (Suharyanto & Nabila, 2021). The acetic acid content in the ecoenzyme also acts as a natural antibacterial that inhibits the growth of pathogens, thus creating a sterile wound environment conducive to granulation tissue formation (Zhang et al., 2020). The pH of the gel, which is in the range of 4.71-4.82, matches the physiological pH of the skin, which is important for maintaining the stability of healing enzymes and proteins. Furthermore, in the remodeling phase, the sterile wound environment and antioxidants in flavonoids help maintain the stability of the new tissue and stimulate collagen maturation. Fibroblast activity becomes more directed in forming minimal scarring. Thus, the combination of antioxidant and antibacterial effects makes ecoenzyme spray gel a topical agent that supports the entire healing process effectively (Mundriyastutik et.al., 2023).

CONCLUSION

Salak pondoh peel ecoenzyme with a concentration of 20% showed the most potent antibacterial activity against *S. aureus* and P. *aeruginosa*, and was most effective in accelerating the healing of burns in male rats within 18 days compared to other concentrations.

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