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The Implementation of FT-IR Method for Compound Detection in Eco-Enzyme Applied as Hydrogel Patch

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Abstract

Potential organic waste includes fruit and vegetable peels which are used as eco-enzyme which are rich in benefits in several fields, one of which is in the health sector, namely hydrogel patch. The aim of this research is to identify the compound content in eco-enzyme and understand the impact of hydrogel patch on the process. healing open wounds. The research method used is the FT-IR method to detect the compound content of eco-enzyme which are applied as hydrogel patchs to heal open wounds in mice. The research was planned to involve 5 treatments in each group. The conclusion in this research is that the presence of flavonoid, alkaloid and tannin compounds in eco-enzyme has been identified and shows that the 5 treatments on eco-enzyme hydrogel patch have concentrations of 25%, 35% and 45% which are stable or 100% recovery, because the results One-Way ANOVA analysis in pre-clinical hydrogel patchs on mice in open wound healing showed a sig value of $p = 0.283 > \alpha$ (0.05). The analysis results show that the functional group (OH) appears at an absorption of 3293.56 cm^{-1}, at an absorption of 2174.49 cm^{-1} it shows the presence of an Alkyl Nitrile functional group, the Amide functional group (CN) is found at an absorption of 1636.34 cm^{-1} and at an absorption of 572.03 cm^{-1} there is an active functional group Trialkyl (CS) sulfide.

Keywords: organic waste, eco-enzyme, hydrogel patch, diabetes mellitus, FT-IR

INTRODUCTION

In Indonesia, organic waste production reaches 84,607.68 tons/day or 30,911,430.20 tons annually. Household organic waste is one of the largest contributors to waste in Indonesia, contributing 40.91% of the total quantity of waste, 17.35% more than the volume of waste arising from traditional markets. According to the Ministry of Environment and Forestry, the largest type of waste is food waste, which reaches 39.81% of total waste, higher than plastic waste at 17.7% (Aloysius et al., 2023)

The characteristics of waste involve the physical, chemical, and biological properties inherent in the waste (Triviño-Pineda et al., 2024). Waste from landfills (TPA) allows for natural processes that can create new life, conditions in which organisms thrive. Recycling waste produces biogas, electricity fertilizer, and various other environmentally friendly products(Bijang et al., 2018). So, eco-enzyme is a concrete example of efficient and environmentally friendly implementation in utilizing organic waste (Hajam et al., 2023)

Eco-enzyme a multi-purpose liquid produced by fermenting organic kitchen waste, such as fruit and vegetables, with the addition of molasses and clean water. Dr. Rosukon Poompanvong-Thailand who has conducted research for 30 years in the development of eco-enzyme. According to him, eco-enzyme is a liquid that has millions of benefits and is a scientific solution to replace toxic synthetic chemicals at home (Nurfajriah et al., 2021)

Several research studies show the benefits of using eco-enzyme in everyday life, such as researchers who found that processing eco-enzyme into soap has proven to be more effective in killing bacteria compared to liquid eco-enzyme. The results of the arganoleptic analysis in the research showed that 56.25% of the antiseptic quality was at a good level, 43.95% was at a very good level, and 9.06% stated that the quality of antiseptic soap was considered sufficient. Eco-enzyme has various benefits such as a disinfectant and hand sanitizer for health purposes, it can also be applied to children to reduce infections and allergies, as well as speed up the healing process (Rusdianasari et al., 2021). So, there is inspiration or innovation to use eco-enzyme as a wound-healing hydrogel patch.

Wound patches that are commonly sold and distributed still have weaknesses, such as a lack of elasticity and causing an uncomfortable sensation on the skin when replaced with a new one, resulting in new wounds forming and the wounds getting bigger (Chamidah & Rohmawati, 2022). Thus, we need a wound patch that has more effectiveness, namely hydrogel, where hydrogel is a synthetic polymer material that is hydrophilic but insoluble, such as poly (methacrylate) and polyvinyl pyrrolidone. The abundant water content in the hydrogel (70-90%) encourages the growth of granulation tissue and epithelium in a humid environment. Hydrogel can reduce the temperature of skin wounds, therefore providing a calming effect and cooling sensation on skin wounds (Grota, Betihavas, Burston, & Jacob, 2022)

The characteristics of an optimal wound patch are that it creates an environment or conditions that have a level of humidity that supports wound healing, does not allow contamination by other microbes, is hypoallergenic, has antimicrobial or antibacterial properties, and can heal wounds according to Parubak & Herawati (2022). The main standards for wound patch include being non-toxic, not causing allergic reactions, having adequate mechanical characteristics, having sufficient strength, and also being elastic. In terms of application, wound patches can be classified into primary dressings, namely patches that come into direct contact with the wound and secondary dressings that are used after covering the main wound. To minimize the occurrence of amputations in diabetes mellitus sufferers (Dudhamal, 2023).

Diabetes mellitus is a chronic disease as a metabolic disorder that shows an increase in blood sugar levels that exceed the optimum limit (Hartmeier et al., 2022). According to the International Diabetes Federation, in 2022, 537 million adults aged 20-79 years will suffer from diabetes globally. Estimates suggest this number increasing to 643 million (1 in 9 adults) by 2030, with continued growth to reach 784 million (1 in 8 adults) in 2045. Diabetes causes 6.7 million deaths in 2021. It is estimated that 44% of adults with diabetes do not receive a diagnosis (240 million people). Global data obtained from 541 million adults or 1 in 10 faces the body's inability to tolerate increased glucose in the blood or often called hyperglycemia which can increase the risk of developing diabetes (Gaspersz & Sohilait, 2019). The Ministry of Health of the Republic of Indonesia noted that the accumulation of people affected by diabetes mellitus reached 19.47 million people (Tao et al., 2024)

By referring to the problems from previous research, researchers experimented with using FT-IR to detect compound content in eco-enzymes which were applied as hydrogel patches for healing open wounds in mice. There are not many studies that know the potential for eco-enzyme content in the form of hydrogel patch, so this research is very relevant as an alternative material to review this problem. The use of variations in the concentration of hydrogel patch on eco-enzyme aims to analyze the characteristics of the gel preparation produced and determine the optimal concentration of the eco-enzyme solution which is very suitable for healing open wounds or 100% recovery.

METHODOLOGY

Materials and Instrumentals

The tools used to make eco-enzyme are a digital scale, pH meter, jar, plastic, stirrer, knife, and cutting board. Other production tools for testing and analysis include analytical balance (Kern-German model: ADB 200-4), hot plate stirrer (Cole Parmer), oven, watch glass, glass beaker (Herma), Erlenmeyer (Iwaki) thermometer (GEA medical S -006), stainless steel spatula, Pyrex stir bar, dropper pipette (ONEMED), 100x15mm petri dish (Iwaki), glucometer (Sinocare), oral probe (Odsidi medical), 1 mL BD Syringe syringe with needle, and FT-IR Spectrophometer brand Nicolet iS10.

The organic waste components used for the eco-enzyme manufacturing process are obtained from Bojonegoro traditional market waste in the form of star fruit, oranges, pears, apples and vegetables, namely carrots, Chinese cabbage, cabbage, and green beans.

The raw materials used to make eco-enzymes are organic waste from fruit peels, vegetables, molasses, and water. Other raw materials used for formulation and testing are PVA (polyvinyl alcohol), NaCl (natrium chloride), PEG 400 (polyethylene glycol), HPMC (hydroxypropyl methylcellulose), alloxan (Persada Scientific Source), and mice.

Methods

Making Eco-enzyme

The main samples for making eco-enzyme are fruit peel waste (apples, pears, star fruit, oranges) and vegetables (carrots, cabbage, white mustard) obtained from the Bojonegoro modern market. Sorted, then fermented for 3 months. The first thing to do is (1) Prepare the tools and materials needed, (2) Fruit and vegetables that have been collected and separated, cleaned in running water and cut into small pieces, (3) Weigh the molasses, fruit and vegetable peel waste so that get the desired ratio of 1:3 in the research, (4) Provide water, vegetable fruit peel waste, and water with a ratio of 1:3:10 to start the next process, (5) Add molasses and water, then stir until dissolved, (6) Add the vegetable fruit peel waste, then stir until evenly distributed and close the container tightly, (7) After one month has passed, open the lid of the container, then stir evenly to ensure a homogeneous mixture, (8) After three months, the eco-enzyme solution separated from the dregs. The eco-enzyme produced is then subjected to phytochemical screening.

Eco-enzyme Phytochemical Screening (Ramadani et al., 2022)

Flavonoid Test

1 mL sample of eco-enzyme was put into a test tube, and then 2 mg of magnesium (Mg) powder and 3 drops of 37% HCl were added. Positive test if red, yellow, or orange color is formed.

Alkaloid Test

1 mL sample of eco-enzyme was put into a test tube, then reacted with 5 drops of Dragendorff's reagent. A positive test is indicated by the formation of an orange color on the sample.

Tannin Test

1 mL sample of eco-enzyme was put into a test tube, then reacted with 5 drops of 1% $FeCl_3$. A positive test is indicated by the formation of a blackish-blue or blackish-green color.

Triterpenoid Test (Irianto et al., 2023)

1 mL sample of eco-enzyme was put into a test tube, and then 2 drops of $CHCl_3$ solution were added. Next, 3 drops of Lieberman Burchard's reagent were added to the mixture. A positive test is indicated by the formation of a purple-red ring.

Functional Group Identification with FTIR (Widjanarko, et al., 2023)

Analysis of eco-enzyme liquid samples using FTIR was carried out to identify functional groups and certain bonds in eco-enzyme. Identification is determined based on the infrared wave absorption band on the sample. Infrared wave absorbance was measured with an FTIR spectrophotometer.

Making Hydrogel Patch (Hanistya et al., 2021)

Aquadest is heated to a temperature of 200 °C, and then hydroxypropyl methylcellulose (HPMC) is poured into a glass beaker filled with distilled water at a speed of 200 rpm. Then add sodium chloride (NaCl) until homogeneous for 20 minutes, then pour in polyvinyl alcohol (PVA) for 20 minutes, once homogeneous, polyethylene glycol (PEG 400) plasticizer is added and the eco-enzyme liquid is stirred for 30 minutes. Leave it at normal temperature for 10 minutes, then put it in the oven at 100°C for 2 hours.

Hydrogel Patch Specification Test (Hanistya & Samlan, 2021)

Organoleptic Testing.

Organoleptic observations include examining the shape and color of the resulting hydrogel patch.

Patch Thickness Measurement.

Measure the thickness of the patch in each formula by measuring the thickness of the patch using a micrometer.

Water Absorption Measurement (Purwasih et al., 2023)

After the patch was stored at room temperature in a desiccator for 24 hours, an initial weighing was carried out to measure its weight as initial weight (Wt), then it was placed in the oven at 100°C, left for 2 hours, then weighed again to determine the weight change. Percent moisture absorption is calculated as final weight (wf) using equation 1.

$$\% \text{ Moisture absorption} = \frac{Wf - Wt}{Wt} \ x\ 100\% \quad (1)$$

Treatment of Experimental Animals (Andika et al., 2023)

Induction and administration of alloxan.

The total number of mice used was 20 mice grouped into 5 groups, with each group consisting of 4 mice. Each part was placed in a cage that had been labeled, and then weighed to see the initial body weight of the mice. Before being given treatment, the mice were fasted for 24 hours (the mice were still given water). Then blood samples were taken from the tip of the mice's tails to determine the initial sugar level before administering alloxan. Mice with a body weight of approximately 20 gr were given orally with an alloxan dose of about 0.5 mg. After 1 day, blood samples were taken to determine the increase in sugar levels. The same thing was done on day 3 to check the sugar levels in mice using One-Way ANOVA analysis.

Pre-clinical test method for hydrogel patch on mice.

The day before the wound was given, the mice had their fur shaved on the back area, then cleaned the back area using 70% alcohol. Then the mice were anesthetized using chloroform by inhalation. Next, an incision was made on the mice using a scalpel with a size of 1 cm and a depth of 0.2 cm. Then the incision wound is cleaned using an alcohol solution, then covered with a hydrogel patch. The hydrogel patch was replaced once every 3 days for 14 days. Wound area healing was measured using One-Way ANOVA analysis and observing changes in the diameter of open wounds in male mice and wound color on days 1, 3, 7, 10, 14 during the replacement of the hydrogel patch.

Data Analysis

The analysis methodology applied is One-Way ANOVA, to identify the impact of the treatment applied.

RESULTS AND DISCUSSION

Results of Making Eco-enzyme

Eco-enzyme known as a fermented liquid made from organic ingredients including fruit, vegetables, molasses, and water. The use of eco-enzyme in hydrogel patches can be linked to developing more environmentally friendly and sustainable formulations. During the fermentation process, chemical transformations occur that involve changes in certain molecules (Benny et al., 2023). After the fermentation process reaches the perfect stage, the formation of eco-enzyme (dark brown liquid), aromatic with alcohol with an acidity level (pH) of 4.0 occurs. In (Figure 1) it can be seen that the final result of the eco-enzyme manufacturing process also forms suspended sediment at the bottom of the container consisting of vegetable and fruit remains, as is known that in eco-enzyme there is one component of the compound chemicals, namely acetic acid which is also known as ethanoic acid with the chemical formula CH_3COOH and pure alcohol, which has antiviral, antibacterial and antimicrobial properties. Meanwhile, the enzyme content includes lipase, trypsin, amylase which can kill or prevent the growth of pathogenic bacteria (Wikaningrum & Anggraina, 2022).

Figure 1. Eco-enzyme

Phytochemical Screening

The results of qualitative tests carried out on eco-enzyme samples aim to determine the secondary metabolites contained in them. The results of the eco-enzyme screening can be seen in (Table 1) which shows that eco-enzyme contains flavonoids, alkaloids and tannins. However, the eco-enzyme liquid did not show any triterpenoid content.

Table 1. Fluid Phytochemical Screening Results Eco-enzyme

Test	Reactor	Results	Information
Flavonoids	Mg + HCl 37%	+	red, yellow or orange
Alkaloids	*Dragendorff*	+	orange
Tannin	$FeCl_3$ 1%	+	Blackish green
Triterpenoids	$CHCl_3$ + Lieberman Burchard	-	orange

Information:(+) identified compound class; (-) compound class not identified.

Based on the test results of several testers, which can be seen in (Figure 2), several positive tests were obtained. The eco-enzyme liquid was tested with Mg powder reagent added with 37% HCl showing positive results with a color change to red, yellow, or orange. This condition shows that the eco-enzyme liquid contains flavonoid compounds, the Dragendorff reagent to test for the presence of alkaloid compounds gave positive results by forming an orange color, and the 1% $FeCl_3$ reagent showed positive results due to a blackish-green color change. Meanwhile, reagents $CHCl_3$ added with Lieberman Burchard showed negative results because there was no color change.

Figure 2. Eco-enzyme phytochemical screening results

The presence of flavonoid content in the eco-enzyme produced (Table 1) is because the fermentation process in the eco-enzyme preparation can produce new compounds. This also happened in previous research, namely fermented fresh vegetables such as pakchoy and mustard greens contain more flavonoids than fresh ingredients (Irianto et al., 2023). Tannin is a secondary metabolite of the polyphenol group which can function as an antifungal, namely Aspergillus niger, Aspergillus flavius, and Candida albicans, while as an antibacterial, namely Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium. Through the iron (III) chloride solution reagent, the presence of tannins in the eco-enzyme can be identified. The phenol group in tannin will react with Fe^{3+} ions from $FeCl_3$ to form a blackish-green complex compound (Ramadani et al., 2022)

Eco-enzyme Function Group

FTIR analysis is used to determine the functional groups and covalent bonds of a material. Determining the functional groups of a molecule or material can estimate the outline of the bioactive compounds contained in a material, including eco-enzyme materials.

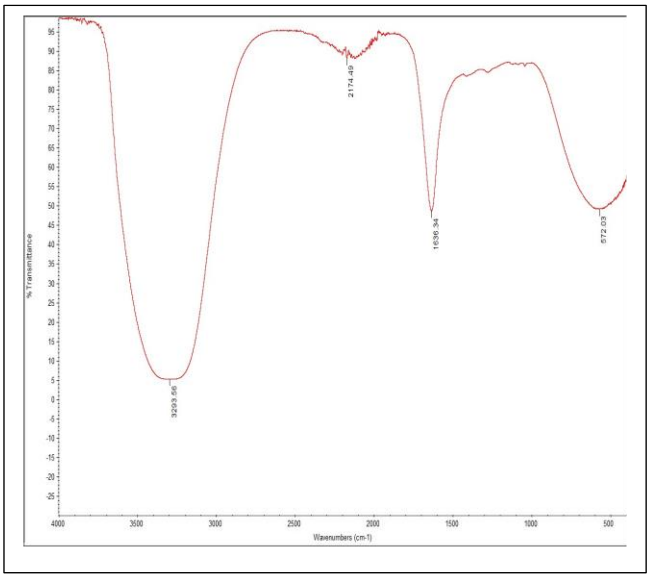

Figure 3. FT-IR analysis Eco-Enzyme

The FTIR analysis can be seen in (Figure 2) and (Table 2). The functional group (OH) at an absorption of 3293.56 cm^{-1}. This strengthens the suspicion that carboxylic acid compounds that are rich in -OH active groups can act as a source of nutrition for microorganisms that are beneficial for the growth of good bacteria and other microbes that are useful in various contexts and have antimicrobial properties, which can help in controlling the growth of pathogenic microorganisms.

Table 2. Functional Groups and FTIR Spectrum Absorption Eco-enzyme.

Wave number (cm-1)		
FTIR Standard	**Eco-enzyme isolation**	**Information**
3294.42	3293.56	OH
2190	2174.49	Alkenes
1640	1636.34	Amida
597.14	572.03	Trialkyl CS organic sulfides and aliphatic disulfide

The absorption of 2174.49 shows the presence of the Alkyne functional group (C≡C) which helps increase skin moisture and helps prevent infection and reduce inflammation in the wound area, so it can provide a faster therapeutic effect. In the FTIR spectrum absorption 1636.34 which shows the presence of the amide functional group (CN), the lipase enzyme activity is measured in the spectrum absorption 1600-1700 cm^{-1}, where the primary amide group band will appear. The lipase activity in eco-enzyme allows this liquid to be used for several human purposes such as liquid soap, disinfectant, gutter cleaner, and antibacterial (Soleha et al., 2023). The absorption spectrum of eco-Enzyme at 572.03 cm^{-1} shows the active group of Trialkyl CS organic sulfide and aliphatic disulfide, with a weak signal. This shows that eco-Enzyme is a clear liquid product with a fresh aroma, a liquid with a complex composition containing disulfide organic compounds.

Results of Making Hydrogel Patch

Hydrogel patches with control variations (hydrogel patches without eco-enzyme) and hydrogel patches with eco-enzyme concentrations of 15%, 25%, 35%, and 45% involve the formulation process of certain ingredients to produce products with different characteristics. The hydrogel patch in the control group was made from hydrogel base material without the addition of eco-enzyme with the result being a standard hydrogel patch which has the natural water absorption capacity of hydrogel.

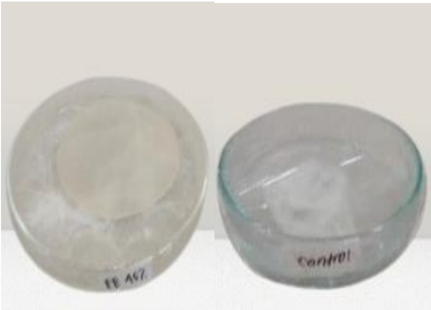

Figure 4. Hydrogel Patch

Hydrogel patch with eco-enzyme liquid concentrations of 15%, 25%, 35%, and 45% in the formulation. Eco-enzyme, which is an enzyme-rich solution from fermented organic waste from fruit and vegetable peels, can provide additional properties in the hydrogel. The higher the concentration of eco-enzyme given to the hydrogel patch, the higher the antimicrobial, antibacterial, and anti-inflammatory potential so that it regenerates damaged tissue. The maximum or faster healing effect

Hydrogel Patch Specification Test Result

The organoleptic test results (Table 1) of each hydrogel patch with a predetermined eco-enzyme concentration have the same shape and color, namely round and slightly brown, except for the patch used as a hydrogel patch without eco-enzyme (control) which has a clear color. From the hydrogel patch thickness test results, the lowest average value shown in the table is 2.5 mm for eco-enzyme hydrogel patch with concentrations of 25%, 35% and 45% compared to hydrogel patch without eco-enzyme (control) has a value of 2.8 mm and 15% eco-enzyme patch with a value of 2.7 mm. The results of the hydrogel patch moisture absorption test in the table show different values from the results of hydrogel patch without eco-enzyme (control) with an absorption capacity of 1.4%, hydrogel patch with an eco-enzyme concentration of 15% with an absorption capacity of 1.8%, hydrogel patch 25% concentration with an absorption capacity of 2.5%, hydrogel patch 35% concentration with an absorption capacity of 2.7%, hydrogel patch 45% concentration with an absorption capacity of 2.86%. In its application, the high water absorption capacity of hydrogel is very useful for absorbing exudate from wet wounds, and the relatively large water content is useful for providing moisture and a cooling effect on wounds.

Results of Data Analysis of Male Mice

Table 2 shows that measuring the average glucose data in mice can be done to understand the influence or effect of a particular treatment or condition on glucose levels in the mice's blood. Based on the graph in Figure 3, it can be concluded that the average glucose levels in mice when fasting are around 75 mg/dL, 68.75 mg/dL, 79 mg/dL, 65.25 mg/dL, and 81.25 mg/dL. dL which shows that glucose levels are still normal. In mice, glucose levels induced with alloxan averaged around 159.25 mg/dL, 126mg/dL, 165mg/dL, 120.75mg/dL, and 171.5mg/dL, thus showing a significant increase in glucose in diabetic conditions. On the 3rd day after administering alloxan, glucose levels in mice averaged around 185.75 mg/dL, 180.75 mg/dL, 196 mg/dL, 178.5 mg/dL, and 216.5 mg/dL. which shows that the mice's glucose exceeds the normal limit so that they develop type 1 diabetes, which involves a lack of insulin. Data obtained from research on blood glucose levels of male mice were then tested for normality to see whether the data was normally distributed or not. If the data meets the normality requirements, then homogeneity is tested to see whether the data variants are homogeneous or not, then proceed to analysis of variance.

Table 3. Hydrogel Patch Specification Test Results

Patch Variations	Organoleptic Testing	Patch Thickness Measurement	Water Absorption Measurement
Patch without eco-enzyme (control)	Round, clear	2.8mm	1.62%
Eco-enzyme hydrogel patch 15%	Round, slightly brown	2.7mm	1.8%
Eco-enzyme hydrogel patch 25%	Round, slightly brown	2.5mm	2.5%
Eco-enzyme hydrogel patch 35%	Round, slightly brown	2.5mm	2.7%
Eco-enzyme hydrogel patch 45%	Round, slightly brown	2.5mm	2.86%

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Table 4. Glucose Levels of Male Mice

Variation	Fast	Induction	Day-3	Total
Patch without Eco-enzyme (control)	75	159.25	185.75	420
Eco-enzyme patch 15% concentration	68.75	126	180.75	375.5
Eco-enzyme patch 25% concentration	78	165	196	439
Eco-enzyme patch 35% concentration	65.25	120.75	178.5	364.5
Eco-enzyme patch 45% concentration	81.25	171.5	216.5	469.25

Table 5. Wound Diameter Data for Male Mice

Variation	H+1	H+4	H+7	H+10	H+14	Total
Patch without Eco-enzyme (control)	1	0.9	0.6	0.4	0.4	3.3
Eco-enzyme patch 15% concentration	1	0.8	0.7	0.4	0.35	3.25
Eco-enzyme patch 25% concentration	1	0.8	0.4	0.1	0	2,3
Eco-enzyme patch 35% concentration	1	0.8	0.3	0	0	2.1
Eco-enzyme patch 45% concentration	1	0.7	0.1	0	0	1.8

Table 3 shows the effect of hydrogel patch without eco-enzyme (control) and variations in eco-enzyme concentration of 15%, 25%, 35%, and 45% of the diameter of the cut wound in mice whose development was observed by observing the diameter of the open wound which has been presented in the graph. On observation on the 4th day, it was seen that the diameter of the open wounds in the control group and the open wounds in the 15% eco-enzyme treatment were larger than the open wounds in the 25%, 35%, and 45% concentration eco-enzyme treatments. The intensity of the white-red color of the wound was both a result of control treatment and administration of eco-enzyme in large concentrations. On the 7th day of observation, the color of the open wound in the control group and the 15% concentration of eco-enzyme became reddish and the diameter of the open wound became wider, whereas in the 25%, 35%, and 45% concentration of eco-enzyme the color of the open wound changed to red with intensity. moderate and the diameter of the wound decreases. Observing the wound on the 10th day, changes were showing that the diameter of the open wound became smaller on the eco-enzyme hydrogel patch with concentrations of 25%, 35%, and 45%. Observing open wounds on the 14th day, there were signs of healing in the open wounds with hair growth and color changes approaching stable or normal.

Hypothesis testing uses One-Way ANOVA. The hypotheses to be tested include; 1) There is an effect of alloxan on increasing blood glucose levels in mice, and 2) Pre-Clinical hydrogel patch on mice in healing wounds in mice (wound diameter in male mice). The results of the One-Way ANOVA test are in (Table 4). Based on the analysis, it was found that the sig p value = 0.005 < α (0.05), indicating that there was a very significant difference in the average value of blood glucose levels in male mice between the five treatment variation groups. Therefore, population variance exhibits a significant degree of non-uniformity. The results of the One-Way ANOVA test on pre-clinical hydrogel patch on mice in wound healing in mice (wound diameter in male mice) are shown in (Table 5). Based on the analysis, it shows that the wound healing time data in each group provides a normality test resulting in a sig p value = 0.283 > α (0.05) which indicates that the distribution of wound healing time data in the three treatment groups is normal.

Table 6. Test results *One-Way ANOVA Glucose in Male Mice*

	Sum of Squares	Degrees of Freedom	Square Mean	F	Sig
Between Groups	2,548,483	4	637,121	206,545	0.005
Within Groups	1,315,994,876	10	131,594,487		
Total	1,318,543,358	14			

Table 7. Test results *One-Way ANOVA of Wound Healing*

	Sum of Squares	Degrees of Freedom	Mean Square	F	Sig
Between Groups	0.376	4	0.094	3,358	0.283
Within Groups	39,904	120	0.3325		
Total	40.28	124			

CONCLUSION

The results of the phytochemical screening of the Eco-enzyme liquid contain flavonoid, alkaloid and tannin compounds, so its use in the form of a hydrogel patch is stable or 100% recovery, and has quite high antibacterial activity in wound healing which shows the results of the One-Way ANOVA analysis in pre-clinical hydrogel patches on mice to heal open wounds, namely sig $p = 0.283 > \alpha$ (0.05).

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