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Test Effectiveness of Ethanol Extract Ointment Papaya Leaves (*Carica Papaya L.*) Against Healing of Cut Wounds on White Rabbit

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ABSTRACT

Wound is a state of damage to body tissue caused by animal bites, scratchessharp objects. A rapid wound healing process is expected. Papaya leaf is effective as a wound healer, the use of extracts directly on the skin is not practical and optimal. In order to be practical and effective, it is formulated in the form of ointment preparations. This study aims to determine the effectiveness of papaya leaf (Carica papaya L.) ethanol extract ointment preparation in healing cut wounds in New Zealand white rabbits made in the form of ointment preparations. The extract was given with different concentrations of 5%, 10% and 15%. The ointment preparation was tested on test animals with a wound length of 2 cm. The positive control used was povidone iodine, while the negative control used ointment bases namely adeps lanae and vaseline album. The data were analyzed statistically using ANOVA (Analysis of Variant). Based on the results of the research that has been done, the concentration of papaya leaf ethanol extract ointment that is most effective in healing cuts is at a concentration of 15%.

INTRODUCTION

Wounds are a condition of tissue damage that is influenced by certain factors such as trauma, animal bites, scratches from sharp objects and others. Based on the cause, wounds are divided into 2 types, namely open wounds and closed wounds (Bawotong et al., 2020). Cuts (incision wounds) are damage that occurs to skin tissue due to trauma from sharp objects such as knives, razors, sharp axes, or swords. When body tissue is injured, there are several effects that arise such as bleeding and blood clotting, loss of all or part of

organ function, bacterial contamination, sympathetic stress response, and cell death (Galomat et al., 2021). One of the preparations as a wound healer is by using a cream, gel, wound healing ointment. The use of thick extracts directly on the skin is impractical and not optimal, so it is necessary to make a preparation that can stick to the skin surface for a long time, is occlusive so that it is effective in healing wounds, namely a semi-solid preparation in the form of an ointment (Tamuntuan et al., 2021).

Traditional Indonesian plants have the potential as wound healing agents. Based on data from the National Workshop on Medicinal Plants in 2010, out of 30,000 types of plants in Indonesia, 940 types are very efficacious as medicine (Nurrani, et al., 2015). One of these medicinal plants is Carica papaya L. In Indonesia, Carica papaya L. is better known as the papaya plant (Setyani, 2016). Papaya leaves contain several substances such as saponins which are compounds that form collagen in the process of wound healing, in addition, papaya leaves also contain beta carotene, Vitamin C and Vitamin E which act as antioxidants that can neutralize free radicals in the wound healing process, and papaya leaves also contain an enzyme papain which can help accelerate the work of macrophages by increasing the production of interleukins which function in the wound healing process (Ricky et al., 2018).

METHOD

The research conducted in this study was an experimental research type, aimed at obtaining observational data on the use of papaya leaf (Carica papaya L.) ethanol extract ointment preparations made for healing cuts in New Zealand White rabbits.

Tool

The tools used in this study were rotary evaporator (Buchi rotary evaporator R-114), analytical scales, glassware (pyrex), 60 mesh sieve, water bath (B-One), hair clipper, dropper, scalpel, porcelain cup, pH meter, blender, ointment pot, cotton and gloves.

Material

The materials used in this study were papaya leaves (Carica papaya L.), 96% ethanol, vaseline album, adeps lanae, male rabbits, Mayer's reagent, Wagner, Dragendroff, 10% povidone iodine, and 1% FeCl3.

Research Procedures

1) Plant Determination

   Identification of papaya leaves (Carica papaya L.). Plant determination aims to ensure the identity of the plants that will be used as research materials so as to avoid errors in sampling.

2) Making Simple Drugs

   Fresh papaya leaves are obtained by wet sorting, and washing, slicing, drying and dry sorting, to the storage stage. Drying by drying under the sun covered with black cloth until all parts are dry.

3) Making papaya leaf ethanol extract

   500 g of papaya leaf powder is macerated with 5 liters of 96% ethanol solvent until the papaya leaf simple powder is put into the macerator with stirring, namely 3 x 24 hours.

4) Preparation of Ointment Preparations

   Preparation of Papaya Leaf Extract Ointment (Carica papaya L.) is made with the following formulation of 100 g:

   Adeps lanae 15 g
   Vaseline Album 85 g
   mf ointment 100 g
   (Agoes, 2006).

   The ointment preparations used had different concentrations of papaya leaf extract, namely 5%, 10% and 15% made in 100 g quantities.

5) Physical evaluation of papaya leaf ethanol extract ointment preparation

   1. Organoleptic Test

      Visual observations include the shape, color and smell of the ointment preparation that has been made (Paju et al., 2013).

   2. Homogeneity Test

A little ointment is taken and then applied to a piece of object glass or other transparent object. In the application results, homogeneous ointment is characterized by the absence of lumps, a flat structure and has the same color from the starting point to the end point (Paju et al., 2013).

3. Spread Power Test
   The sample was weighed as much as 0.5 g then placed in the middle of the scaled glass and above the ointment was placed another round glass or other transparent material and a weight so that the weight of the round glass and weight was 150 g, left for 1 minute, then the diameter of the spread was recorded. The good spreadability of the ointment is between 5-7 cm (Bessie et al., 2018).
4. pH Test
   The pH test is carried out using a pH meter by dipping it into the preparation, which has previously been calibrated with a buffer solution. After the color changes, it is compared with the pH color on the packaging. In accordance with the pH value of human skin, a good pH value for ointment ranges from 4.5 -7 (Paju et al., 2013).
5. Adhesion Test
   As much as 0.5 grams of ointment is placed on a predetermined object glass. Another object glass is placed on top of the ointment. After that, a 50gram load is added for 1 minute on the object glass and then released and the time is recorded until the two object glasses are released (Rahmawati et al., 2010)
6. Viscosity Test
   The ointment preparation is put into the ointment pot, then spindle no. 4 is installed and the rotor is run at a speed of 6 rpm. The viscosity value of the ointment preparation is in the range of 2000-50000 cP (Kawarnidi et al., 2022).
7. Hedonic Test
   Observations were made using Organoleptic tests on the color, aroma, and texture of the ointment preparation made from papaya leaf ethanol extract with 20 panelists.
8. Cut Wound Testing
   The healing activity used healthy male white rabbits aged 2-3 months with a weight of 1-1.5 kilograms as many as 4. Before making the wound, the rabbits were acclimatized for 5 days with the aim that the test animals were able to adapt to the new environment and treatment so that the test animals were not stressed. The day before making the wound, the fur of the test animals was shaved on the back area until smooth. The shaved area was cleaned with 70% alcohol and then rested for 24 hours. The next day, each marked part was then cut using a scalpel with a length of 2 cm on the back and a depth of about 0.1 mm. Application of ointment (F1: 5% extract ointment, F2: 10% extract ointment, F3: 15% extract ointment, F4: Negative Control and F5: Positive Control) on each cut wound was carried out three times a day (every 8 hours). The negative control used was an ointment base without extract, while the positive control used was 10% povidone iodine ointment.
9. Data analysis
   The data obtained were analyzed using SPSS (Statistical Package for The Social Science) to determine the effectiveness of papaya leaf (Carica papaya L.) ethanol extract ointment preparation. Statistical tests used normality test, homogeneity test and One Way Anova (Analysis of Variance) test.

RESULTS AND DISCUSSION

1. Plant Determination

This research began with the determination of plants. The results of the determination showed papaya leaves (Carica papaya L.).

2. Organoleptic Test

Organoleptic observation is a physical parameter to observe changes in shape, color and odor.

Table 1. Organoleptic test results

Formulation	Color	Smell	Form
Negative Control	Light yellow	Odorless	Semi Solid
FI	Dark green	Papaya Leaf Specialty	Semi Solid
F2	Dark green	Papaya Leaf Specialty	Semi Solid
F3	Dark green	Papaya Leaf Specialty	Semi Solid

Information:
Negative Control: As an ointment base without extract
FI: Papaya leaf extract ointment 5% concentration
FII: Papaya leaf extract ointment 10% concentration
FIII: Papaya leaf extract ointment 15% concentration

3. Homogeneity Test

Homogeneity Test is an important requirement for good pharmaceutical preparations, homogeneity testing is carried out to observe the composition of the preparation in a homogeneous form and there is no clumping. Based on the results obtained in each formula concentration of 5%, 10% and 15% and negative control showed good homogeneity properties and no coarse particles were seen when viewed with a glass object.

Table 2. Homogeneity test results

Formulation	Homogeneity
Negative Control	Light yellow
FI	Dark green
F2	Dark green
F3	Dark green

Information:
Negative Control: As an ointment base without extract
FI: Papaya leaf extract ointment 5% concentration
FII: Papaya leaf extract ointment 10% concentration
FIII: Papaya leaf extract ointment 15% concentration

4. Adhesion Test

Table 3. Adhesion test results

Formulation	Adhesion force(seconds)	±SD
Negative Control	1.79	0.7
FI	3.21	0.7
F2	3.23	0.7
F3	3.29	0.7

Information:
Negative Control: As an ointment base without extract
FI: Papaya leaf extract ointment 5% concentration
FII: Papaya leaf extract ointment 10% concentration
FIII: Papaya leaf extract ointment 15% concentration

Based on the results obtained, the higher the concentration, the higher the adhesive power, the results obtained meet the requirements for good adhesive power with a range of 1 - 4 seconds.

5. Spread Power Test

The purpose of the spreadability test is to determine the softness of the ointment mass so that the ease of applying the preparation to the skin can be seen. Based on the data obtained, the results meet the requirements for good spreadability, which is 5 cm. The requirements for an ointment preparation with good spreadability are 5 - 7 cm. A good ointment preparation can spread easily on the surface of the skin without using pressure (Sugiyono, 2019).

Table 4. Spread Power Test Results

Formulation	Adhesion force(seconds)	±SD
Negative Control	5.2	0.05
FI	5.2	0.05
F2	5.3	0.05
F3	5.2	0.05

Information:
Negative Control: As an ointment base without extract
FI: Papaya leaf extract ointment 5% concentration
FII: Papaya leaf extract ointment 10% concentration
FIII: Papaya leaf extract ointment 15% concentration

6. Viscosity Test

This Viscosity Test aims to see the thickness of an ointment preparation. Viscosity is carried out using a Brookfield tool. The viscosity value of the ointment preparation is in the range of 2000 - 50000 cP. In formula I there was an increase to formula III, this happened because of the difference in viscosity of each formula after the addition of different extracts.

Table 6. Viscosity Test Results

Formulation	Viscosity Value (cP)
Negative control	8058
Formulation I	8357
Formulation II	8825
Formulation III	8846

Information:
Negative Control: As an ointment base without extract
FI: Papaya leaf extract ointment 5% concentration
F II: Papaya leaf extract ointment 10% concentration
F III: Papaya leaf extract ointment 15% concentration

7. Hedonic Test

Table 7. Hedonic Test Results for the Aroma Section

	Aroma			
	F0	**F1**	**F2**	**F3**
	59	81	64	56
Average	2.95	4.05	3.2	2.8

Table 8. Results of the Hedonic Test for the Texture section

	Texture			
	F0	**F1**	**F2**	**F3**
	74	73	70	70
Average	3.7	3.65	3.5	3.5

Table 9. Hedonic Test Results for Color Section

	Color			
	F0	**F1**	**F2**	**F3**
	73	75	64	74
Average	3.65	3.75	3.2	3.5

Based on the results of the hedonic test in table 6, the aroma section was obtained in formula 1 with a concentration of 5% getting the highest value, the panelists chose the criteria of liking and the most in formula 1 because the panelists preferred the aroma of formula 1. This is because in formula 1 the aroma of the extract does not smell as pungent as formula 3. Aroma is one of the indicators that influences the panelists' preference for the preparation. The results of the hedonic test in table 7, the texture section, showed that each formula had similarities, namely smooth. On average, respondents chose Formula 0 as the most preferred. In table 8, the color section, it is known that the panelists' assessment of the papaya leaf ethanol extract ointment preparation in formula 1 was the most preferred. The color of the papaya leaves which gives a natural color makes the preparation liked by the panelists.

8. Test the effectiveness of wound healing

Figure 1. Cut Wounds on Rabbits

The effectiveness test of wound healing using papaya leaf ethanol extract ointment used male New Zealand White rabbits as test animals. The reason for choosing male rabbits is because their hormonal condition is more stable compared to female rabbits which will experience hormonal changes during pregnancy and lactation so that the stress level in female rabbits is higher than in male rabbits. Before the effectiveness test of papaya leaf ethanol extract ointment was carried out, an acclimatization process was first carried out on the test animals for 5 days which aimed to allow the test animals to adapt to the environment where the experiment was carried out so that the test animals did not experience stress.

The administration of papaya leaf ethanol extract made into ointment can provide a healing effect on cuts. Povidone iodine 10% as a positive control to determine the equivalence of the effects given by the ointment preparation in determining the most effective concentration of papaya leaf ethanol extract. Testing the effectiveness of healing cuts is characterized by a reduction in the length of the cut for 14 days.

CONCLUSION

Based on the results of papaya leaf ethanol extract (Carica papaya L.) can be made a good ointment preparation, and meets the criteria of a good ointment. Papaya leaf ethanol extract ointment preparation (Carica papaya L.) has

effectiveness in healing cuts in New Zealand White rabbits. The best concentration of papaya leaf ethanol extract (Carica papaya L.) in the form of ointment preparation that can heal cuts in New Zealand White rabbits is at a concentration of 15%.

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