



## The Effectiveness of Ethanol Extract *Stachytarpheta jamaicensis* towards Cut Wounds in *Mus musculus*

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### ABSTRACT

The plant *Stachytarpheta jamaicensis* is an annual herbaceous shrub. Cuts are wounds that occur due to cuts by instruments or sharps objects. This study aims to determine the effectiveness of the ethanol extract *Stachytarpheta jamaicensis* on the healing of cuts in *Mus musculus* and the most effective concentration for healing cuts in *Mus musculus*. Design This study uses laboratory experimental methods. The sample used for each experimental group was 5 mice with a total of 5 groups, so this study used 25 mice with 3 treatments, namely the treatment (positive control (+), negative control (-), 25%, 50%, 75%). All data is tabulated and statistically analyzed with ANOVA. The conclusion shows that the concentration of *Stachytarpheta jamaicensis* extract at a concentration of 75% is the most effective in healing cuts in *Mus musculus* when compared to *Stachytarpheta jamaicensis* extract at concentrations of 50% and 25% in terms of the average percentage of healing from day 1 to day 7.

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### INTRODUCTION

Wounds are damage or loss of body tissue that occurs due to a factor that interferes with the body's defense system. According to Arysanty, a wound is a state where the continuity of the body's tissue is interrupted which can cause disruption of bodily functions, so that it can interfere with daily activities.<sup>1</sup>

Wounds consist of several types including incisions or cuts, bruises, abrasions, stab wounds, lacerations and others. In addition, the shape is also different. The shape of the wound is different depending on the cause, some are open and closed. One example of an open wound is an incision. Incisions or cuts are

wounds that can occur on purpose such as surgical wounds or by accident which are referred to as traumatic wounds due to incisions of sharp objects as broken glass, kitchen knives and zinc<sup>2,3</sup>, meanwhile according to Kurniawaty et al.'s cuts are wounds that occur because of being sliced by instruments or sharp objects.<sup>4</sup>

Wound care management is needed to improve all aspects of healing, prevent further skin damage, reduce the risk of infection and increase patient comfort. Various types of wounds associated with the stage of wound healing require proper wound management. Wound care is currently developing very rapidly. In its development, the results of

research on wound care show that a moist environment is better than a dry environment. Plants in Indonesia have many varieties and properties, including as medicinal plants. There are various types of medicinal plants in Indonesia. One of the medicinal plants is *Stachytarpheta jamaicensis*. *Stachytarpheta jamaicensis* contains alkaloids, flavonoids, glycoside derivatives, phenolic derivatives, quinones, saponins, steroids, tannins and terpenoids.<sup>5</sup>

*Stachytarpheta jamaicensis* which have been studied and proven to have several bioactivities such as antibacterial, antioxidant<sup>6</sup>, and the ethanol extract of *Stachytarpheta jamaicensis* have anti-inflammatory and analgesic activities.<sup>7</sup> *Stachytarpheta jamaicensis* have been used empirically as a remedy for allergies, respiratory disorders, colds, coughs, fever, constipation, indigestion, and menstrual disorders.<sup>5</sup> In addition, this plant is usually used by the community as a pain reliever, gastric medicine, sedative, bronchitis and others.<sup>8</sup>

Several others studies have been conducted on *Stachytarpheta jamaicensis* related to the chemical compounds contained, one of which is research conducted by Indrayani, et al.'s, that the chemical compounds contained in *Stachytarpheta jamaicensis* include triterpene compounds, flavonoids, tannins, saponins, have the potential to heal wounds by acting as antioxidants and antibacterial mechanisms.<sup>9</sup> In addition, tannins and triterpenes can act as astringents in wounds while saponins work to increase the speed of epithelialization.<sup>10</sup>

Based on the background above, the researcher is interested in conducting a study entitled the effectiveness of ethanol extract *Stachytarpheta jamaicensis* towards cut wounds in *Mus musculus*.

## MATERIAL AND METHOD

### Research Design

The type of research used was laboratory experimental, namely by grouping horse whip leaf extract (25% 50% 75%) and then giving it to the test animals (mice).

### Place and Time of Research

This research was conducted at the Biology Laboratory of the Medica Farma Husada Polytechnic Mataram from March to June 2021.

### Population and Sample

The population that was used as the object of this study were green *Stachytarpheta jamaicensis* obtained in the yard and roadside located in Jontak hamlet, Central Lombok. The sample in the study were 500 grams of dark green leaves in a dry state.

According to Fredrer, the formula for determining the sample for the experimental test is:

$$\begin{aligned} (t - 1) (n - 1) &\geq 15 \\ (5 - 1) (n - 1) &\geq 15 \\ 4(n-1) &\geq 15 \\ 4n - 4 &\geq 15 \\ 4n &\geq 15 + 4 \\ 4n &\geq 19 \\ n &= 19/4 \\ &= 4.75 \sim 5 \end{aligned}$$

Information: t = is the number of experimental groups.

n = number of repetitions or number of samples per group.

$$\begin{aligned} \text{Correction Factor} &= n / (1 - F) \\ &= 5 / (1 - 0.1) \\ &= 5 / 0.9 \\ &= 5.55 \sim 6. \end{aligned}$$

So the sample that will be used for each experimental group is 5 tails and the number of groups to be used is 5 groups so that this study uses 25 tails.

### Research Instrument

Research instruments are tools or facilities that can be used by researchers in collecting data so that work is easier and the results are better, in the sense that it is more accurate, complete and systematic so that it is easy to process.<sup>11</sup> The instrument used in this research is observation.

### Research Tools and Materials

1. The tools used
  - a. Aluminum foil
  - b. Stirring rod
  - c. Glass beakers
  - d. Knife
  - e. Blender
  - f. Pen
  - g. Mortar
  - h. Filter paper
  - i. Drop pipette
  - j. Gloves

- k. Places to eat and drink test animals
- l. Analytical balance
- m. Glass jar
- 2. Material
  - a. Ethanol 70%
  - b. Stachytarpheta jamaicensis
  - c. Mus musculus (25 Heads)
  - d. Vaseline Album
  - e. Betadin Ointment
- 3. Preparation stage
  - a. Simple setup
    - 1) Collecting Stachytarpheta jamaicensis  
Stachytarpheta jamaicensis found on the side of the road in 1 large plastic bag
    - 2) Selection of Stachytarpheta jamaicensis  
The Stachytarpheta jamaicensis are taken from fresh green leaves
    - 3) Drying Stachytarpheta jamaicensis  
Drying is done in aerated way
  - b. Preparation of tools and materials  
All tools and materials to be used in this study must be washed and then dried
- 4. Manufacture of ethanol extract Stachytarpheta jamaicensis
  - a. The maceration container in the form of a jar is washed, dried and rinsed with ethanol.
  - b. The extract was prepared by maceration using 70% ethanol solvent.
  - c. The leaves obtained were soaked in 70% ethanol
  - d. After that it is stored for 3 days and protected from direct sunlight.
  - e. Then it is filtered with filter paper and the thick extract is taken and evaporated on the stove using a water bath until a thick extract is obtained.
  - f. This process takes approximately 5 hours.
- 5. Making wounds on test animals
  - a. The test animal was shaved on the back of the rat by about 5 cm.
  - b. Making the wound, an incision 2 cm long and 0.3 cm deep was made on the back of the rat.
  - c. After shaving, perform antiseptic action by administering 70% ethanol alcohol
  - d. Perform cleaning by flowing with aquadest until the bleeding stops
  - e. Each group was given the following treatment:
    - 1) Group 1: Cut wounds on Mus musculus were given a negative

control by administering Vaseline Album

- b. Group 2: Mice cut wounds were given a positive control of Betadin Ointment
- c. Group 3: Given 25% Stachytarpheta jamaicensis extract
- d. Group 4: Given Stachytarpheta jamaicensis extract 50%
- e. Group 5: Given Stachytarpheta jamaicensis extract 75%
- 6. Test animal treatment

Giving treatment to each group of test animals, 25%, 50%, 75% of the results of the Stachytarpheta jamaicensis extract on the back skin of rats.

#### Data Collection Technique

Data collection was carried out by means of direct observation and wound care forms. In addition, it also uses photos of wounds for each treatment to reduce subjectiveness in assessing wound healing time.

#### Processing and Analysis of Data

Data analysis in this study used qualitative and quantitative analysis. Qualitative analysis, namely by testing the quality of green betel leaf ointment preparations (organoleptic test, pH test, homogeneity test and skin irritation test of volunteers). Meanwhile, quantitative analysis uses the One Way Anova test.

The data analysis technique used in this study is the normality test with the Kolmogorov test if the sig value is  $> 0.05$  then the test is normal for homogeneity testing can be used. If the results of the homogeneity test are significant, a One Way ANOVA test will be carried out.

#### RESULTS

This research was conducted by collecting green betel leaf materials. Once collected, the betel leaves are chopped and aerated. After the material is completely dry, it is continued with the maceration process with 70% ethanol solvent, stirring is carried out every day until the 3rd day, after which it is filtered using filter paper. The filter results were evaporated with a rotary evaporator in the Monobiology Laboratory, University of Mataram. Then the evaporation results are made into ointment formulations with different concentrations. The treatment of the test animals was started by shaving the hair on the back first, after which a  $\pm 2$  cm incision was given on the back. After being given an incision wound, the length of the

incision was measured and then given 5 treatments, namely: positive control treatment using betadine ointment, negative control using vaseline album, green betel leaf extract concentration of 20%, 50% and 70%. Measurements were taken every day with an interval of 24 hours until the 7th day.

The results of research conducted at the Immonology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Mataram and the Biology Laboratory of the Polytechnic Medica Farma Husada Mataram entitled the effectiveness of the ethanol extract *Stachytarpheta jamaicensis* towards cut wounds in *Mus musculus*.

1. Collection of materials

The *Stachytarpheta jamaicensis* were collected in 1 large plastic bag in one piece, then the selection and washing of the *Stachytarpheta jamaicensis* was chopped and dried in the air for 2 weeks and then after drying. The powdered *Stachytarpheta jamaicensis* that had been blended were

weighed again and 500 grams of *Stachytarpheta jamaicensis* were obtained in a good condition.

2. Fine powder extraction

From 500 grams of dried simplicia, 8 grams of thick extract of *Stachytarpheta jamaicensis* was obtained. After maceration of the filtrate obtained as much as 1 liter then the filtrate was concentrated with (a vacuum rotary evaporator) and obtained as much as 8 grams of viscous extract with a yield of 1.6%.

3. Results of measuring the decrease in the diameter of the incision area

The results of measuring the decrease in the area of the incision wound in the test group with a concentration of 25%, 50%, 75% on day 1 to day 7 based on the data centering method (mean) can be seen in table 4.1.

**Table 4.1. Measurement of Mean Diameter of Cut Area in *Mus musculus* from Day 1 to Day 7**

Day	Positive Control (Betadin ointment)(+)	Negative control (Vaseline) (-)	Stachytarpheta jamaicensis Extract 25% (cm)	Stachytarpheta jamaicensis Extract 50% (cm)	Stachytarpheta jamaicensis Extract 75% (cm)
H0	1,9	1,9	1,84	1,92	1,88
H1	1,6	1,7	1,68	1,6	1,68
H2	1,4	1,6	1,52	1,54	1,5
H3	1,08	1,34	1,34	1,4	1,32
H4	0,8	1,08	1,24	1,3	1,1
H5	0,5	0,68	0,94	1,14	0,6
H6	0,5	0,34	0,56	0,38	0,18
H7	0	0	0	0	0
Average	1,075	0,91	1,14	1,16	1.0325

Based on table 4.1, it can be seen that the average wound healing rate in *Mus musculus* from day 1 to day 7, the highest average was seen in the 75% *Stachytarpheta jamaicensis* extract treatment with an average of 1.0325, while the lowest average was seen in the treatment *Stachytarpheta jamaicensis* extract 25% with an average of 1.14.

**Research Data Analysis**

**Normality Test**

**Table 4.2. Normality Test Results**

Treatment	Kolmogorov-Smirnova <sup>a</sup>		
	Statistic	Df	Sig.
<b>Stachytarpheta jamaicensis</b>			
<b>25%</b>	.240	8	.196*
<b>50%</b>	.190	8	.200*
<b>75%</b>	.163	8	.200*

Normality test A statistical test conducted to find out how the data is distributed.

The results of data analysis obtained from the normality test for *Stachytarpheta jamaicensis* extract are significant. If the significant value obtained is > 0.05, then the

test is homogeneous, then ANOVA testing can be done.

**Homogeneity Test**

**Table 4.3. Homogeneity Test Results**

Levene Statistic	Df1	df2	Sig.
5.247	2	21	.142

Homogeneity test is a test conducted to find out that two or more of the sample data groups come from populations that have the same homogeneous variance.

The results of the data analysis obtained from the homogeneity test for *Stachytarpheta jamaicensis* extract were significant  $142 > 0.05$ . If the significant value obtained is  $> 0.05$ , then the test is homogeneous, then ANOVA testing can be done.

**Anova Test**

**Table 4.4. Anova Test Result**

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3504.52	2	1752.2	4.171	.03
Within Groups	31421.8	23	1496.2		
Total	74		80		
	34926.3				
	95				

ANOVA is a statistical analysis method that includes statistical inference ANOVA used to test the difference in means (average) of data for more than two groups.

The results of data analysis obtained from the ANOVA test for *Stachytarpheta jamaicensis* extract are  $f_{count} (4.171) > f_{table} (3.47)$ . If the value of  $f_{count} > f_{table}$  then the test is significant.

**Table 4.5. LSD Test Results**

(I) Treatment	(j) Treatment	Mean Diffirance (I-J)	Std Error	Sig.	95% Confidance Interval	
					Lower Bound	Lower Bound
LSD Concentration 75%	Concentration 50%	-1075	.3372	.752	-792	.577
	Concentration 25%	-1325	.3372	.697	-817	.552
	Positive control	.1225	.3372	.719	-562	.807
	Negative control	-0425	.3372	.900	-727	.642
Concentration 50%	Concentration 75%	.1075	.3372	.752	-577	.792
	Concentration 25%	-0250	.3372	.941	-710	.660
	Positive control	.2300	.3372	.500	-455	.915
	Negative control	.0650	.3372	.848	-620	.750
Concentration 25%	Concentration 75%	.1325	.3372	.697	-552	.817
	Concentration 50%	.0250	.3372	.941	.660	.710
	Positive control	.1225	.3372	.455	-430	.940
	Negative control	.0900	.3372	.791	-595	.775
Positive control	Concentration 75%	-1225	.3372	.719	-807	.562
	Concentration 50%	-2300	.3372	.500	-915	.455
	Concentration 25%	-2550	.3372	.455	-940	.430
	Negative control	-1650	.3372	.628	-850	.520
Negative control	Concentration 75%	.0425	.3372	.900	-462	.727
	Concentration 50%	-0650	.3372	.848	-750	.620
	Concentration 25%	-0900	.3372	.791	-775	.595

Based on the results of the LSD test, it shows that it is known that the pairwise comparisons with the LSD test show that there are several average pairs that have different averages. These different pairs can be seen in the sig value of the paired test which is smaller than 5%. From the test results it can be seen that the pair of different averages is in the pair of 75% concentration with negative control, 50% concentration and 25% concentration. Thus it can be concluded that the average LSD test

results show that the different averages are at a concentration of 50%, a concentration of 25% and a negative control, while a concentration of 75% and a positive control have the same average.

**DISCUSSION**

Wounds are damage or loss of body tissue that occurs due to a factor that interferes with the body's defense system. These factors



include trauma, temperature changes, chemicals, electric shock explosions, or animal bites. The shape of the wound differs depending on the cause, some are open and some are closed. An example of an open wound is an incision where there is a linear tear in the skin and underlying tissue. In terms of the cause, the wound is divided into two, namely cuts and burns. Cut wounds are wounds caused by sharp objects. This wound has the properties of smooth wound edges, there is no connection between the tissues and no necrotic tissue.<sup>12</sup> An incision wound is a wound that occurs because it is sliced by a sharp instrument, for example occurs as a result of surgery. The characteristics are an open wound, pain, the length of the wound is greater than the depth of the wound.<sup>13</sup> Healing can be seen based on indicators of loss of redness, swelling and closing of the wound.

Wounds are loss or damage to a part of body tissue or damage to a network unit/component, where specifically there is a damaged or missing tissue substance. When a wound occurs, several effects will appear including the loss of all or part of organ function, sympathetic stress response, bleeding and blood clots, bacterial contamination and cell death.<sup>14</sup>

Chemical compounds contained in *Stachytarpheta jamaicensis* include triterpene compounds, flavonoids, tannins, saponins, which have the potential to heal wounds as antioxidants and antibacterials. In addition, tannins and triterpenes also act as astringents in wounds, while saponins work to increase the speed of epithelialization.

In this study, dilution was carried out for each concentration (25%, 50%, 75%) by making a preparation of 8 ml for healing for 7 days. At the time of dilution, there was an inhomogeneity between the extract and the diluent (70% ethanol), so the PEG solvent was added to the extract. In this study, the wound healing test was seen from a decrease in the diameter of the incision area. Treatment of the negative control group had the longest healing effect if the diameter and condition of the incisions were considered, compared to the other treatment groups. This is because the negative control does not contain active substances that can help the healing process of cuts.

The next test is the SPSS test using the Levene test method to obtain homogeneous test results with a significance > 0.05. It can be seen

in table 4.4 that the data is significant > 0.05 in each treatment, H0 is rejected and H1 is accepted so it can be concluded that the data is normally distributed. However, in table 4.5 Anova test with SPSS, 353 > 0.05 for each treatment, there is no significant difference between the average group counts. This is because the concentration used does not vary.

From the results of the SPSS test it was stated that the data were normally distributed and homogeneous, but after being tested with the ANOVA test the results were not significant. This could be due to the concentration range being made too close.

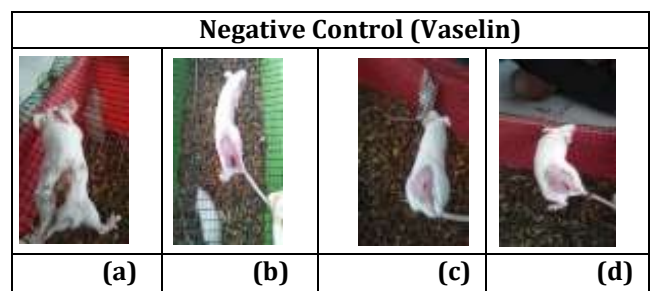


Figure 1. Observation of t Wound Healing

Observation of the effectiveness *Stachytarpheta jamaicensis* extract was carried out by looking at changes in the length of the infected wound (made about 2 cm). Observations were made when there was an infection wound in the form of erythema (redness). Furthermore, the reduction in the length of the diameter of the incision was seen.

From the results of the study obtained data on changes in the length of the incision by calculating the average change in length of the incision with an interval of measurement every 24 hours.

Based on the research that has been done, it is obtained an overview of *Mus musculus* that have been tested for 7 days. On day 1, the percentage of wound healing in the 25% *Stachytarpheta jamaicensis* extract concentration group was 0.2 cm, the 50% *Stachytarpheta jamaicensis* extract concentration group was 0.2 cm, the 75% *Stachytarpheta jamaicensis* extract concentration group was 0.3 cm. Meanwhile, the positive control group showed almost the same percentage of healing with a concentration of 75%, namely 0.3 cm and 0.2 cm in the negative control group.

The reduction in the length of the incision in *Mus musculus* occurred every day until the 7th day, but for the positive control, healing occurred on the 6th day marked by the closing of the incision in the wound.

From the results of the research that has been done, it can be concluded that *Stachytarpheta jamaicensis* extract is effective in healing cuts in *Mus musculus*. In several treatments, the concentration of *Stachytarpheta jamaicensis* extract with a concentration of 75% had a faster wound healing power when compared to *Stachytarpheta jamaicensis* extract with a concentration of 50% and 25%, seen from the average percentage of healing from the 1st to the 7th day. This is because *Stachytarpheta jamaicensis* with a concentration of 75% contain more active substances than 25% and 50%.

## CONCLUSION AND RECOMMENDATION

### Conclusion

Based on the research that has been done, it can be concluded that:

1. *Stachytarpheta jamaicensis* can heal cuts in *Mus musculus*.
2. The effectiveness of *Stachytarpheta jamaicensis* extract with a concentration of 75% is greater than *Stachytarpheta jamaicensis* extract with a concentration of 50% and 25%.

### Suggestions

Suggestions that can be given based on the research that has been done is that it is necessary to carry out further studies regarding the extract concentration intervals. It is better for further research to use more parasitive intervals so as to obtain significant results.

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## AUTHOR CONTRIBUTIONS

Following are the contributions of each author in the research to the completion of this article "Conceived and designed the experiments by Z and BAA; Z performed the experiments; Z and EP analyzed the data; Z and WRR contributed reagents/materials/analysis tools". Z = Zohroyani; BAA = Baiq Ayu Aprilia; EP = En Purmafitriah; Z and WRR = Wulan Ratia Ratulangi".

## CONFLICTS OF INTEREST

There is no conflict of interest in writing this article.

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