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TESTING THE ACTIVITY OF THE N-HEXANE FRACTION OF THE ETHANOL EXTRACT OF BETTER BETTER LEAVES (Momordica Charantia L.) WITH VASELINE CARRIER ON THE HEALING OF CUT WOUNDS IN WHITE RATS (Rattus Norvegicus)

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ABSTRACT

Wound are a form of damage to anatomical structure and function. When a wound occurs, the integrity of the epithelial structure of the skin is lost and dysfungtion occours in the skin. Therefore, normalization of integrity is required as soon as possible. Bitter melon leaves have benefits for wound healingand repairing skin granulation tissue. to understand the most optimal concentration level of N-Hexane Fraction of Bitter melon leaf ethanol extract in healing white rat incision wound using vaselin carrier and to understand the healing activity of N-Hexane fraction of Bitter melon leaf extract on white rat incision wound healing. The research conducted was an experimental type with the test animal, namely 18 white male rats. The white rats were grouped into 6 groups, where the standard control was group I, the positive control was group II with (injuring and giving sagestam cream) treatment, the negative control was group III with (injuring and giving it vaseline base) treatment, the last group IV, V, and VI were treated (injuring and giving it n-hexane fraction of bitter melon leaves at concentration levels of 1,5%, 3%, 6% and the base was vaseline).Then induced an incisions wound 2.0 cm deep and 0.2 cm long, but before shaving the rat's back first and then anesthetizing using lidocaine injection. The observation process is carried out for 2 weeks and data collection is every 2 days. Furthermore, conduct statistical test when the data has been collected. Based on the results of observations and testing of the N-hexane fraction of bitter melon leaf ethanolextract, it has activity in healing cuts in white rats and the most optimal concentration in healing cuts is a concentration of 6%. The N-Hexane fraction of ethanol extract of bitter gourd leaves has activity in healing cuts in white rats and the most optimal concentration in healing cuts is 6%.

Keywords : bitter gourd leaves, N-Hexane fraction, Slice wound.

INTRODUCTION

Wounds are categorized as a type of injury that humans often suffer. When a wound occurs, the epithelial structure of the skin is damaged, causing the skin to be unable to carry out its normal function. In order for the skin to function again, treatment efforts are needed which aim to repair damage to the structure of the skin epithelium (Rinawati et al, 2015). There are three main stages in the wound recovery process based on Theoret's (2017) explanation, including the remodeling (maturation), proliferation and inflammation stages.

Treatment using drugs or herbs is currently increasingly preferred by Indonesian people. One effort to increase safety in the use of herbal medicines is that more research is needed to explore information regarding the properties of herbal plants in Indonesia (Dalimartha, 2012).

One type of vegetable that is often consumed by Indonesian people is Momordica Charantia L, or known as the bitter melon plant. If we look more broadly, in Southeast Asian countries, China and Brazil, this plant is widely used as traditional medicine (Poolperm S, 2017; Magalhaes et al., 2019).

According to research data from Rusmin et al., (2020) and Sudarsi (2018), empirically bitter melon leaves are widely used to treat wounds, sore throats, dysentery, coughs, diabetes mellitus and fever. Other research by Anjum F. (2012), and Yoshime (2016) shows that bitter melon plants in a pharmacological context act as anti-hepatotoxic, antiviral, antimicrobial, anti-cancer and anti- diabetic. There are several research results related to the efficacy of bitter melon leaf extract as a wound healer, namely, the first is the result of research by Ananda R. et al. (2020) explained that burns that occurred on rabbits' backs could heal after being smeared with bitter melon leaf extract. This is because bitter melon leaf extract has the potential to heal wounds because it contains secondary metabolite compounds such as terpenoids, steroids, alkaloids, saponins and flavonoids. Second, the research results of Muhamad Pazry et al. (2017) showed that the injured backs of male mice could recover because they were given bitter melon leaf extract.

Based on this literature study, researchers see the potential to develop research regarding the level of effectiveness of the N-Hexane fraction of the ethanol extract of bitter melon leaves as a healing tool for cuts in white rats.

The aim of the research is to understand the level of effectiveness of the N-Hexane fraction of the ethanol extract of bitter melon leaves (Momordica Charantia L.) as healing cuts in white rats (Rattus norvegicus L.) using a vaseline base.

METHODS

A. Collection of Test Materials

Banyuwangi Regency, East Java is the location for collecting bitter melon leaves (Momordica CharantiaL.) used in research. Fresh bitter gourd plants were taken using a random sampling method of 11 kg from several places in the local area. Maintaining the Integrity of the Specifications..

B. Progress of the research

The materials needed for the research are PAM water, pellets, male rats, sagestam® cream (gentamycin cream), chloroform, N-Hexane, Vaseline, 2% lidocaine, 96% ethanol, and bitter melon leaves. Furthermore, to support the implementation of research, the tools needed include masks, gloves, analytical scales, rulers, plaster, syringes, scalpels, scissors, cotton wool, rat food bowls, wire nets, rat cages, dropper pipettes, measuring cups, grinding machines. , and rotary evaporator. The research will take place from March-May 2022 at the Pharmaceutical Laboratory of the STRADA Institute of Health Sciences.

1. Plant identification. The plants tested were identified at the UPT herbal materia medica batu laboratory. Identification was carried out to ensure that the plant under study was definitely bitter melon (momordica charantia L.).
2. Preparation of materials. 11 kg of fresh bitter melon leaves were then sorted and then washed using a drying oven at 45°C for 3 days. The dried sample is then weighed and air-dried. The clean leaf samples were then weighed to obtain the fresh sample weight. After weighing, the plant is chopped into smaller pieces, then dried again to obtain the dry weight, then ground using a grinding machine.
3. Making bitter melon leaf extract. The extract was made using the maceration method using a ratio of 1:10. 3000 grams of bitter melon leaf powder were weighed, then placed in a dark jar and then soaked in 3000 ml of 96% ethanol for three days, shaken twice a day. The maceration container is stored at room temperature and protected from direct sunlight. Next, the maceration results are then

filtered and then evaporated using an evaporator until a thick extract is obtained. The extract was then concentrated using an evaporation oven at a temperature of 40°C. The thick extract obtained is then weighed, the yield and drying loss obtained are recorded, then stored in a cooling room for further thickening.

$$\% \, Yield = \frac{\text{extract weight x 100\% } simple}{weight}$$

$$\% \text{ Drying shrinkage} = \frac{(dry \; simplicia \; weight) \times 100\%}{Beay \; extract}$$

4. Preparation of n-hexane fraction of bitter melon leaves. Weighed 10 g of ethanol extract then diluted using 5 ml of 96% ethanol. Add 70 ml of distilled water then add 75 ml of N-Hexane after placing it in a separating funnel and then shake it gently until the two solvents are mixed. Next, let it sit for 2-3 minutes until it forms two layers. Collect the top layer (N-Hexane), then replicate three times. The n-hexane fraction obtained was then evaporated using an evaporation oven for 24 hours to obtain a thick n-hexane fraction.

5. Identify chemical ingredients using the TLC method. The TLC method was implemented in testing the N-Hexane Fraction of the ethanol extract of bitter melon leaves. Based on the explanation (Fikaamilia H.et al., 2020) the comparison of the mobile phase used is chloroform: ethyl acetate: formic acid (7.5:6:0.5) with the stationary phase of Agel 60 silica plate.

6. Test the wound healing effect. The test animals that will be used before being induced with cuts are subjected to an ethical test by the Health Research Ethics Committee of the Indonesian Strada Institute of Health Sciences. After 18 male white mice passed the ethical test, the next stage was divided into 6 groups, where each group consisted of 3 white mice. The six groups were treated differently, namely negative control, positive control, normal control, 1.5% dose control, 3% dose control, 6% dose control. Next, shave the rats' backs with a diameter of approximately 3 cm after cleaning. with 70% alcohol. Then the incision was induced but first the rat's back was anesthetized by administering a 2% concentration of 2% lidocaine injection in 2 ml via the intramuscular route. Then the section is incised with a depth of 0.2 cm and a length of 2.0 cm using a sterile scalpel No.11. Carry out these steps on the 15 test animals while the other 3 test animals are in the normal control group without induced injuries. The test preparation of the n- hexane fraction of bitter melon leaves was applied topically to the wounds of mice. The normal control group was the group without treatment. The positive control group was smeared with sagestam® cream (gentamycin cream) and the negative control group was smeared with vaseline base. Each wound in each test group was smeared with n-hexane fractions with different concentrations, namely 1.5%, 3% and 6% using a vaseline carrier. The duration of treatment is 14 days. The length of the wound area was measured with the help of a ruler and then the data was analyzed statistically using the SPSS 12 computer program.

RESULTS

A. Results of plant identification. The plants identified at the UPT Batu Herbal Materia Medica Laboratory proved that the plant used was bitter melon (Momordica Charantia L.) with the determination key 1b-2a-27a-28b-29b-30b: cucurbitaceae-1a-2b-3b: Momordica-3: m Charantia.

B. Results of making bitter melon leaf simplicia. As a result of drying bitter melon leaves for 3 days at a temperature of 45°C, 7.6 kg of dry bitter melon leaf simplicia was obtained from 11 kg of fresh bitter melon leaf weight. Next, the simplicia is ground into powder using a grinding machine. The results of pollinating dried simplicia bitter melon leaves obtained a powder weight of 5 kg.

C. Results of making bitter melon leaf extract. The results of making ethanol extract of bitter melon leaves using a ratio of 1:10 resulted in 500 grams of thick extract. In the next stage, the extract obtained is calculated by calculating the percent yield and drying loss. The results of calculating the extract yield obtained a percentage of 10%, which based on the requirements in the Indonesian Herbal Pharmacopoeia, the yield is said to meet the requirements if it is not less than 7.2%. The results of drying loss calculations also obtained a value of 1.5%, where this percentage still meets the maximum limit regarding the amount of compound lost, namely no more than 11% (Ministry of Health of the Republic of Indonesia, 2000).

Results of making n-hexane fraction of bitter melon leaves and TLC test. 10 g of ethanol extract was then diluted using 5 ml of 96% ethanol. Add 70 ml of distilled water then put into a separating funnel then add 75 ml of N-Hexane then shake gently until the two solvents are mixed then let stand for 2-3 minutes until it forms two layers. Collect the top layer (N-Hexane), then replicate three times. The N-Hexane fraction obtained was then evaporated using an evaporation oven for 24 hours to obtain a thick N-Hexane fraction. The result was 150 grams of the n-hexane fraction of the ethanol extract of bitter melon leaves. Next, carry out a phytochemical test using the TLC method to identify the chemical content in the n-hexane fraction of the ethanol extract of bitter melon leaves. Based on the identification carried out, it is obtained:

Tabel 1. Results of chemical content identification using the TLC method

Chemical compounds in the N-Hexane fraction of bitter melon leaves	**Color results after spraying**		**information**
	254 nm	**366 nm**	
Flavonoid	Greenish yellow	Greenish yellow	+
Alkaloid	Orange brown	Orange brown	+
Saponin	Light blue	Light blue	+
Terpenoid	Purplish red	Purplish red	-

The TLC (thin layer chromatography) method was implemented to identify the chemical content of the N-Hexane fraction of the ethanol extract of bitter melon leaves. The F254 silica gel plate was used as the TLC plate. At the fraction spotting stage, use a capillary tube from the bottom edge of the plate at a distance of ± 1 cm. Next, it is dried and the length of the stain movement from the spotting point for each compound is read. If the level of movement reaches the maximum threshold, stop the elution. Then illuminate the surface of the plate with stains using UV light with a wavelength of 254 nm and 366 nm.

The final stage is to observe the stain.

D. Test results for the healing effect of cut wounds

Tabel 2. Results of measuring wound healing in mice

Group Treatment	T0	T1	T2	T3	T4
Normal	0	0	0	0	0
Negative	2.00±0.00	2.00±0.00	1.87±0.15	1.90±0.10	1.77±0.21
Positive	2.00±0.00	1.67±0.58	1.17±0.29	0.17±0.29	0.00±0.00
Kons. 1.5%	2.00±0.00	2.00±0.00	1.33±0.58	1.33±0.58	1.33±0.29
Kons. 3%	2.00±0.00	2.00±0.00	2.00±0.00	1.23±0.40	0.83±0.58
Kons. 6%	2.00±0.00	0.90±0.17	0.83±0.29	0.67±0.29	0.67±0.58

DISCUSSION

The healing process for cut wounds generally begins with the occurrence of erythema. When redness (erythema) occurs, the arterioles widen. According to Rinawati et al., (2015) the cause of red wounds is increased blood flow (vasoconstriction) of the arteries in the damaged tissue. At the same time, an inflammatory process occurs. Macrophage cells in the body will naturally synthesize collagen to cover wounds by producing fibroblasts and angioblasts.

Based on observations on rat cuts, the erythema process occurred on day 0 to day 2. The rat's back after being induced by a cut experienced redness and swelling in the area induced by the wound. On the 3rd day, the wound swelling in several mice in the positive group and the 6% concentration test group began to decrease. The flavonoid compounds in the N-Hexane fraction are thought to have an anti-inflammatory role so they can reduce the inflammatory effect on mouse wounds. Based on the explanation of Negara et al (2014), there are several mechanisms that inhibit the occurrence of inflammation in mouse wounds by flavonoids, including secreting lysosomal enzymes as inflammatory mediators to suppress the proliferation of endothelial cells, neutrophil cells and inflammatory cells, slowing down the performance of arachidonic acid metabolism cyclogenase, slowing down the secretion of histamine and serotonin gets involved in inflammation and slows capillary permeability.

In the next stage, the process of proliferation or fibroplasia occurs. Proliferation generally appears in the late stages of the inflammatory process. In this phase, in order to adjust the tension of the wound when it wrinkles, the collagen that has formed is destroyed. The contracting nature of tilmio fibroblasts causes the wound to be pulled so that later the intramolecular and intermolecular binding remodeling stage becomes stronger and wound closure will occur. In the fibroplasia phase, there is release and movement of basal cells (wound edge epithelium) to the wound surface as a filler. The migration stage stops when the wound is closed and there is touch between the epithelial cells. The process of closing the wound surface as a sign of the fibroplasia phase will stop and then enter the final phase, namely remodeling (Siamsuhidajat R. 2012).

The proliferation process in rat wounds is caused by the presence of saponin compounds in the N-Hexane fraction of bitter melon leaves. The proliferation phase in mouse wounds can be observed from day 7 to day 10. The proliferation process is characterized by the appearance of new skin tissue or scar tissue in the wound. The formation of scar tissue can be observed in the results of measuring rat wounds (T3), namely in the 6% and 3% dose control groups and the positive control group. According to Syamsuhidayat (2014), the formation of collagen can be triggered by saponin compounds which play a direct role in increasing the epithelialization process of skin tissue so that the wound closing process can be faster. The process of proliferation and tissue formation takes 3-14 days (Reddyetal., 2012).

The remodeling stage is the final stage of wound recovery which is characterized by the formation of new skin tissue which has gone through the maturation and shrinkage stages. If the scar has disappeared, it means this stage is over and usually lasts for a matter of months. In the process of healing skin tissue, the body will naturally normalize the damage (Syamsuhidrajat, 2012).

Based on the results of observations on days 12 and 14 (T3 and T4), the remodeling process in each treatment group was not perfect because there were still scars on the mice's backs. In mice in the positive control group and the test group at concentrations of 1.5%, 3% and 6%, the remodeling process was still not perfect. Based on research conducted by Rienda and Susianti (2016), the remodeling process takes months or even years. This is influenced by the depth and level of skin tissue damage experienced.

In the test results, it was found that very significant data on wound healing was shown in the positive control group which used the sagestam cream preparation. Based on the results of the Kolmogorof-Simirnov test regarding the percentage of healing power of incision wounds for 14 days, the results of the normality test were obtained, namely that the data was normally distributed because it had a significance value exceeding 0.05. The test results using One Way ANOVA obtained a significance value of 0.000, which means the P value <0.05, so H0 is rejected and H1 is accepted so that there is a significant difference between each test group. In research conducted by Anom P. et al (2019), the sagestam cream preparation had a good healing effect because it contained gentamycin cream. Even though the cuts in the positive control group had closed, there were still scars (keloids).

In the test group, the N-Hexane Fraction, the ethanol extract of bitter melon leaves, concentrations of 1.5%, 3% and 6% respectively provided a healing process effect. However, the best healing percentage was shown by the test group of the N-hexane fraction of bitter melon leaf extract with a concentration of 6%. The results of statistical tests using Kolmogorov–Smirnov regarding the percentage of healing power of incision wounds in mice during 14 days of treatment obtained a value of sig>0.05, which means the data is normally distributed. Furthermore, the statistical test results using One Way ANOVA (Analysis of Variant) obtained a value (sig 0.000) which means the P value < 0.05, so H0 is rejected and H1 is accepted so that there is a significant difference between the five treatment groups of test animals. The results of the ANOVA test can then be continued for the LSD test where the test results show a significance value of <0.05, which means that H0 is rejected and there is a significant difference between the five rat treatments in each group. These results also show that the administration of suggestam cream, as well as the N- Hexane with concentrations of 1.5%, 3% and 6% both provide healing effects on rat cut

CONCLUSION

The conclusion that can be drawn based on the results of a 14 day research set is that the N-Hexane fraction of the ethanol extract of bitter melon leaves has the activity of healing cuts in white rats and the most optimal concentration of the N-Hexane fraction of the ethanol extract of bitter melon leaves in healing cuts in white rats is the N-Hexane with a concentration of 6%.

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