ANTIBACTERIAL TEST OF BINAHONG LEAF EXTRACT OINTMENT (*Anredera cordifolia*) to *Staphylococcus aureus* BACTERIA FROM DIABETES WOUNDS

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ABSTRACT

Diabetes mellitus (DM) is characterized by an increase in glucose levels in the blood due to disorders of glucose metabolism in the body. The pancreas organ of people with DM has a weakness in producing the hormone insulin. As a result, the distribution of blood glucose to other organs of the body is inhibited so that glucose levels in the blood increase which causes DM sufferers to experience longer wound healing than normal humans. Binahong leaves contain alkaloids, saponins, and flavonoids which have antibacterial activity which can accelerate the wound healing process. The purpose of this study was to determine the antibacterial effect of binahong leaf extract ointment (*Anredera cordifolia*) against *Staphylococcus aureus* bacteria from diabetic wounds. This research includes antibacterial test for binahong leaf extract ointment (*Anredera cordifolia*) with variations in the concentration of 10%, 20%, and 30% of the concentration of binahong leaf extract of 25% which resulted in a wound coverage percentage of up to 100%, namely at a concentration of 30% ointment. For negative control, there was no wound closure and positive control in the form of oxytetracycline, the percentage of wound closure was 85%.

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A. INTRODUCTION

Diabetes mellitus (DM) is characterized by an increase in glucose levels in the blood due to disorders of glucose metabolism in the body. The pancreas organ of people with DM has a weakness in producing the hormone insulin. As a result, the distribution of blood glucose to other organs of the body is inhibited so that glucose levels in the blood increase (Zychowska et al., 2013). So that DM sufferers experience longer wound healing than normal humans. This is because wounds in DM are included in chronic wounds due to the extension of the wound healing phase, namely *haemostasis*, inflammation, proliferation, and *remodeling* (Nagori & Solanki, 2011).

Improper handling of chronic wounds with DM will result in infections which are generally resolved by amputation (Karri et al., 2015). This causes people to look for alternatives in the treatment of diabetes wounds, one of which is herbal plants. The use of herbal plants is increasingly favored by the community with the *trend back to nature*. The middle to lower class communities use a lot of ingredients from natural ingredients, especially in preventive, promotive, and rehabilitative efforts to cope with various diseases (Mutiarra et al., 2015). Herbal plants are currently experiencing rapid development, one of which is binahong (*Anredera cordifolia*) which is believed by the Indonesian people as a medicine that can accelerate wound healing.

The binahong plant (*Anredera cordifolia*) from the *Basellaceae* tribe has an influence in accelerating the healing of skin wounds seen from the effect of cell proliferation (Takahashi et al., 2012) because binahong leaves contain alkaloids, saponins and flavonoids which have antibacterial activity (Rochani, 2009). Saponins have the ability to act as an antiseptic, which kills or prevents the growth of microorganisms that usually occur in wounds so that the wound does not experience severe infection. Flavonoids have a strong effect as anti-oxidants, stimulate the production of nitrite oxidation which can dilate blood vessels and can inhibit fibroblast growth, and can induce cell proliferation so that it can accelerate wound healing (Robinson, 1995).

Khunaifi (2010) has conducted research on the efficacy of binahong leaves against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria which states that the minimum inhibitory concentration of binahong leaf ethanol extract (EEDB) against *Staphylococcus aureus* is at a concentration of 25% which is equivalent to 250 mg / mL, and for *Pseudomonas aeruginosa* bacteria at a concentration of 50% is equivalent to 500 mg/mL. The minimum kill concentration of binahong leaf ethanol extract against *Staphylococcus aureus* is at a concentration of 50% equivalent to 500 mg/mL, whereas for *Pseudomonas aeruginosa* bacteria at 100% concentration is equivalent to 100 mg/mL. Likewise, the research of Paju et al., (2013) produced an effective binahong leaf ethanol extract ointment as a wound medicine due to infection with *Staphylococcus aureus* bacteria at a concentration of 20%.

Based on the research background above, the writer wants to continue the research by making topical use of pharmaceutical preparations, namely ointments, using binahong leaf extracts with various concentrations of active substances and to test the effectiveness of antibacterials, *in-vivo* testing is carried out. Ointment preparations were chosen because ointments have a function as a carrier for topical drugs, as a skin lubricant and as a skin protector. It is hoped that by making binahong leaf extract ointment preparations will make the use of binahong leaves practically usable by people with diabetes wounds.

B. METHOD

This research was conducted at the Microbiology Laboratory of Mataram Hospital and the type of this research was experimental research with a quantitative approach. While the research design used a completely randomized design (CRD) with various concentrations of binahong leaf extract (*Anredera cordifolia*) of 25%, 50%, 75%, 100% and a positive control of Oxytetracycline with 4 replications.

The research procedure consisted of 2 stages, namely the preparation and implementation stages. The preparation stage consists of providing binahong, making binahong leaf extract, isolating *Staphylococcus aureus* bacteria from diabetic wound sufferers, and making wounds on test animals.

Faction Preparation

The fractionation was preceded by making binahong leaf ethanolic extract with ethanol 96% solvent and extracted by reflux method.

Isolation of *Staphylococcus aureus* bacteria from diabetic wound sufferers

To get the *Staphylococcus aureus* bacteria from people with diabetes wounds, it must be done to remove the wound from the sufferer. After getting the wound smear with a cotton swab, immediately put it in *Physiological Na* solution before putting it in MSA (*Medium Salt Agar*) medium and incubated for 1 x 24 hours. The results of the isolation from the media are then multiplied on NAP media (*Nutrient Agar Plate*) and gram stain is done.

Antibacterial Activity Test of Binahong leaf extract

To determine the ability of binahong leaf extract as an antibacterial in inhibiting the growth of pathogenic bacteria, an in-vitro test was carried out to determine the size of the zone of inhibition. The equipment and materials used for the inhibition zone test are first sterilized by autoclaving. The working method is carried out in a sterile manner in a laminar air flow room to prevent contamination. Sensitivity test performed using the wells and dripped 10 mL binahong leaf extract, then dripped on pitting media Mueller Hinton order that had been inoculated with the bacteria *Staphylococcus aureus* isolated from patients with diabetes mellitus as much as 0.1 mL of the bacterial density equivalent to 1 McFarland (3×10^8 cells/mL). Each treatment at each concentration was repeated 4 times, then incubated for 24 hours at 37 °C in an incubator. The diameter of the resistance zone generated in this test is then.

Making Wounds on Test Animals

Wound making in rats the rat's hair on the back was shaved off and made a 5 mm diameter wound using a punch biopsy. The wound preparation is initiated with anesthesia using ketamine 0.2-0.3 mL intramuscularly.

Testing the effectiveness of binahong leaf extract ointment on infected wounds.

Observations were carried out for 24 hours to see if the wound was swabbed with *Staphylococcus aureus* bacteria. After the infection was observed, it was observed for 5 days to heal the infected wound. Observation of infected wounds was carried out before administration and after treatment until there were signs of healing by measuring the length of the infected wound. Each mouse was treated as follows:

- Treatment A : Media containing *Staphylococcus aureus* bacteria was based on ointment (Negative Control).
- Treatment B : Media containing *Staphylococcus aureus* bacteria was given oxytetracycline skin ointment (Positive Control).
- Treatment C : Media containing *Staphylococcus aureus* bacteria was given 10% binahong leaf extract ointment.

- Treatment D : Media containing *Staphylococcus aureus* bacteria was treated with 20 % binahong leaf extract ointment .
- Treatment E : Media containing *Staphylococcus aureus* bacteria was treated with 30 % binahong leaf extract ointment .

Percentage of Wound Cover (PL%)

The percentage of wound closure is calculated using the formula:

$$P = \frac{d_o - d_n}{d_o} \times 100\%$$

Note:

d_o = Diameter of the initial wound

d_n = Wound diameter on the day of observation

The diameter was obtained from the average of two wound areas on the right and left back by measuring 4 sides of the wound diameter with a caliper and from the measurement of the wound diameter was carried out in the afternoon.

C. RESULTS AND DISCUSSION

The results of testing the antibacterial activity of ashitaba leaf extract using the sumuran method showed that the binahong leaf extract (*Anredera cordifolia*) had antibacterial activity as shown in the following **Figure 3.1**.

Figure 3.1. Inhibition of Binahong Leaf Extract Against Growth of *Staphylococcus aureus* Bacteria from Diabetic Wounds

Based on the picture above, it can be seen that binahong leaf extract (*Anredera cordifolia*) can inhibit the growth of the *Staphylococcus aureus* bacteria isolated from diabetic wounds. This is characterized by a clear zone around the well. This is shown in **Table 3.1**.

Table 3.1. Average Diameter of Inhibition Zone (MM) Results of Antibacterial Activity Test of Binahong Leaf Extract (*Anredera Cordifolia*) Against *Staphylococcus Aureus* Bacteria from Diabetic Wounds

No	Treatment (Concentration)	The average diameter of the inhibition zone (mm)
1	Positive Control	31,4
2	25 %	15,4
3	50 %	18,4
4	75 %	20,4
5	100 %	22,4

Table 3.1 shows the diameter of the inhibition zone starting to form at a concentration of 25% and the diameter of the inhibition zone continues to increase at a concentration of 100%. This is because binahong leaves contain alkaloids, saponins and flavonoids which have antibacterial activity (Rochani, 2009). Whereas for positive control using oxytetracycline also had a larger inhibition zone when compared to binahong leaf extract. However, long-term use of antibiotics will result in bacteria becoming resistant to these antibiotics. Testing Binahong leaf extract ointment standardization includes parameters of identity, organoleptic, homogeneity, and pH.

Table 3.2. Results of Testing of Binahong Leaf Extract Identity and Organoleptic Parameters

Parameters	Results
Shape	Thick extract
Color	Dark brown
Smell	Aromatic
Taste	Bitter

Table 3.3. Homogeneity test results Binahong Leaf Extract Ointment

Parameters	Results
There are no coarse particles and nothing clot	Homogen

The homogeneity test aims to see and determine the mixing of ingredients ointment preparations. Based on the results of the above observations, it can be concluded that the ointment preparation is made evenly, i.e. on each side there are no coarse particles and has uniform particles on the glass slide used during the test, so that the results of the ointment preparation made meet the physical quality parameter requirements, namely the ointment preparation. must be homogeneous.

The pH test aims to determine the safety of the ointment when used so that it does not irritate the skin and test the pH of the ointment using a pH meter. The results of the observation of the pH test for binahong leaf extract ointment had a pH of 6.6. The pH results of the preparations are in accordance with the SNI requirements which state that the pH range of the ointment preparations that meets the requirements is 3.5-8. Incompatible pH values will cause pH changes and damage to the skin epidermis. Damage to the epidermis of the skin can cause the skin to lose its acidity, become more easily damaged and irritated. Macroscopic wound healing can be seen from the reduction in wound diameter or wound coverage percentage parameters as shown in the following table.

Table 3.4. Percentage of *Mus musculus* Sp wound closure with Binahong Leaf Extract Ointment, Vaseline, and Oxytetracycline Ointment Treatment

Day	Day Percentage of wound closure				
Number	Vaseline (Negative control)	Oxytetracycline (Positive control)	10% ointment	20% ointment	30% ointment
1	0	71.2	66.8	68.5	72.3
2	0	76.1	68.8	70.4	80.6
3	0	80.6	72.4	78.9	81.8
4	0	81.2	80.6	82.2	84.6
5	0	85.0	85.2	88.9	100

Table 3.5. LSD Test Results Percentage of Wound Closure

Group Comparison	Sig.
Oxytetracycline : Vaseline	0,000
Oxytetracycline : 10% ointment	0.390
Oxytetracycline: 20% ointment	0.823
Oxytetracycline : 30% ointment	0.286
Vaseline : 10% ointment	0,000
Vaseline : 20% ointment	0,000
Vaseline : 30% ointment	0,000
10% ointment : 20% ointment	0.521
10% ointment : 30% ointment	0.062
20% ointment : 30% ointment	0.201

Table 3.5 above shows that the binahong leaf extract ointment dosage of 30% is the most effective in accelerating wound healing compared to binahong with a dose of 20%, 10%, negative control (vaseline), and positive control (oxytetracycline). Macroscopic wound healing on day 5 is best seen in the treatment of binahong leaf extract ointment concentration of 30% with 100% wound closure percentage and the lowest at 10% concentration with a percentage of 85.2 %%. Another case with the negative control, there was no wound healing in the mice, whereas for the positive control using oxytetracycline ointment, there was 85% wound healing up to day 5.

The 30% concentration of binahong leaf extract ointment has the highest potential in accelerating wound closure. Binahong leaves contain many polar, semi-polar, and *non-polar* chemical compounds that play a role in wound healing such as tannins, saponins, alkaloids, steroids, triterpenoids, flavonoids, and essential oils (Ekaviantiwi, 2013). These compounds play a role in wound healing (Ghosh & Gaba, 2013).

Table 3.6. Tukey Test Results Percentage of wound closure

Observation	Subset for alpha = 0,05	
	1	2
Vaseline	0,000	
10 % Ointment		74,78
20% Ointment		77,78
30% Ointment		78,82
Oksitetrasiklin		83,86
Sig.	0,000	0,313

Table 3.6 above shows that vaseline treatment with ointment (10%, 20%, 30%) and oxytetracycline showed a significant difference. This is because the percentage of wound closure for negative control using vaseline was 0%. Meanwhile, the treatment between ointment (10%, 20%, 30%) and oxytetracycline showed no significant difference. The use of binahong leaf extract ointment has the potential to accelerate wound closure because binahong leaves contain polar, semi-polar, and non-polar chemical compounds that play a role in wound healing such as tannins, saponins, alkaloids, steroids, triterpenoids, flavonoids, and essential oils (Ekaviantiwi, 2013). These compounds play a role in wound healing (Ghosh & Gaba, 2013). On the other hand, using antibiotics in the long term will result in bacteria becoming resistant to these antibiotics.

D. CONCLUSION

The higher the concentration of binahong leaf extract ointment, the higher the antibacterial effect this can be seen from the higher percentage of wound closure against *Staphylococcus aureus* isolated from diabetic wounds because the active compounds in binahong leaf extract are increasing. While the use of antibiotics in the form of oxytetracycline shows the wound closure power is only up to 85% because the potency of antibiotics decreases and even will be lost.

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