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Antibacterial Activity Test and Wound Healing Infection Telang Leaf Extract (*Clitoria ternatea L.*) in Hyperglycemic Rabbits

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ABSTRACT

This study was conducted to determine the antibacterial activity and wound healing of Telang (*Clitoria ternatea L.*) leaf extract against *Staphylococcus aureus* ATCC 25293 and *Escherichia coli* ATCC 25922 in 15 New Zealand rabbits aged 3-5 months weighing 1500–2500 grams of hyperglycemia induced by alloxan and randomly divided into 5 groups, namely normal, negative control (cream base), positive control (Sagestan® which each gram contains Gentamycin Sulfate equivalent to 1 mg of Gentamycin), topical administration of 10% and 20% Telang leaf extract cream. The back skin of the hyperglycemic rabbit was injured with a punch biopsy with a diameter of 5 mm, a depth of ± 0.5 mm, then treated according to the test group for 14 days. Parameters measured included wound diameter and histological observations of infected and non-infected wounds in hyperglycemic rabbits.

The results showed that 10% Telang leaf extract cream had antibacterial activity against *S. aureus* and *E. coli* which was comparable to the positive control, namely the diameter of the inhibition zone against *S. aureus* and *E. coli* was 27.3mm and 18.5mm. Telang leaf extract cream also had infection wound healing activity which was comparable to positive control in hyperglycemic rabbits, wound healing activity for infected wounds was 0.9 mm and non-infected wounds were 0.8 mm.

Keywords: *Clitoria Ternatea L.*, Antibacterial, Wound Healing, Alloxan

INTRODUCTION

The preparation of drugs from natural ingredients is one of the government's discourses in utilizing biological resources. Indonesia is a country that has a large natural wealth but is still underutilized. The telang flower plant (*Clitoria ternatea L.*) has been widely cultivated as an ornamental plant and has even been used for medicine (Fitmawati *et al.*, 2016). Based on previous research, the Telang flower plant has antimicrobial properties (Uma *et al.*, 2009), the antibacterial and antifungal activity test of the Telang flower methanol extract showed an inhibitory effect against *Aspergillus niger* and *Alternaria solani* (Naz *et al.*, 2013) and *Staphylococcus aureus* (Puhane, *et al.*, 2013). Another study, showed that the aqueous extract of the Telang flower parts at a concentration of 5% showed a 46% growth inhibition of *Fusarium oxysporum f.sp. cepae* *in vitro*. Reportedly, Telang leaves contain alkaloid compounds, flavonoids, saponins and steroids (Nurgustiyantri *et al.*, 2021).

Hyperglycemia can be caused due to environmental factors and unhealthy lifestyles, such as overeating, fat, lack of activity, stress and heredity so that this disease is also known as "Mother of Disease" because it is the parent of several diseases, such as hypertension, blood vessels blood, heart, stroke, kidney failure and blindness (Putri *et al.*, 2013).

Infectious wounds most often occur due to poor hygiene and the availability of adequate drugs to speed up the healing process of infectious wounds is still very limited, even though the development of the drug industry is very advanced, so it is necessary to have alternative drugs that can accelerate the healing of open wounds so that infection does not occur (Sandra, 2016). Antibacterial tests can be carried out in several ways, one of which is the diffusion method on agar media that has been inoculated with test bacteria with a diameter of about 5-8 mm which is then filled with a solution or preparation



containing antimicrobial substances and incubated at an optimum temperature of around 37°C. Observations were made by looking at the presence or absence of inhibitory zones formed around the wells. The abundance of plants in Indonesia, the increasing interest in researching the benefits of plants as potential sources of new treatments, the use of telang flower plants is not only limited to coloring food and anti-oxidant drinks. Telang leaf has potential as an alternative to antibiotics because of its antibacterial activity. (Rajesh, et al., 2017). Based on this, the researchers were interested in testing the antibacterial activity and wound healing of the extract of telang leaf extract (*Clitoria ternatea* L.) in hyperglycemic rabbits.

METHOD

A. Materials

The materials used in this study were Telang leaves, *S. aureus*, *E. coli*, NaCl 0.9%, etanol 70%, etanol 9%, HCl 2N, HCl pekat, Sagestan® krim, serbuk Magnesium, pereaksi *Dragendorff*, pereaksi *Liebermann-Bouchard* (asam asetat anhidrat-H₂SO₄), air suling, Kloroform, plat silica, Metanol, Etanol, Butanol, Asam Asetat, *Nutrient agar* (NA), *Vogel Johnson Agar* (VJA), *Endo Agar* (EA), *Mueller Hinton Agar* (MHA), *Brain Heart Infusiom* (BHI), larutan standar *Brown II*, *Heart Infusion Agar* (HIA), *Sulfide Indol Motilitas* (SIM), *Kliger Iron Agar* (KIA), *Lisin Iron Agar* (LIA), *Simmon Citrat*.

B. Setup Simplicity

Telang leaves are washed with running water to remove the existing contaminants, then dried at a temperature of 40°C using an oven, mashed by using a grinding machine until it becomes powder.

C. Making Extract

A total of 1000grams of Telang leaf simplicia powder was macerated with 3000ml 70% ethanol solvent for 72 hours, then the resulting extract was evaporated using a vacuum rotary evaporator. The results of the evaporation were put into the oven at a temperature of 40°C to obtain a thick extract. The results of the concentration were then tested for ethanol free and the extract yield was calculated.

D. Preparation of Alloxan solution

A total of 1000grams of Telang leaf simplicia powder was macerated with 3000ml 70% ethanol solvent for 72 hours, then the resulting extract was evaporated using a vacuum rotary evaporator. The results of the evaporation were put into the oven at a temperature of 40°C to obtain a thick extract. The results of the concentration were then tested for ethanol free and the extract yield was calculated.

E. Formulation of Cream of Telang Leaf Extract

In this study, a Vanishing cream base was used which also functions as a topical carrier base for Telang leaf extract, the composition of the formulation can be seen in table 1.

Table 1^a. Cream formula

No.	Materials	Base Creams	Base Creams	Telang leaf	Telang leaf
		Surfactant + (grams)	Surfactant - (grams)	extract cream 10% (grams)	extract cream 20% (grams)
1	Asam stearat	7,882	7,882	7,882	7,882
2	Glycerin	5,972	5,972	5,972	5,972
3	Na. Riborat	0,149	0,149	0,149	0,149
4	Trietanolamin	0,596	0	0,596	0,596
5	Aquadest	44,798	45,394	39,798	34,798
6	Nipagin	0,012	0,012	0,012	0,012
7	Extract Telang leaf	0	0	5	10

F. Antibacterial Activity Test

The ethanol extract of taro leaves was then tested microbiologically against *S. aureus* and *E. coli* bacteria using the diffusion method to determine antibacterial activity on MHA media. The wells are made using a drill prop and placed on the agar medium which is planted with microorganisms that will diffuse into the agar medium. Positive results can be seen in the clear area on the surface of the media which indicates the inhibition of bacterial growth by antibacterial agents (Pratiwi 2008).

G. Data Analysis

Data analysis using SPSS, namely normality and homogeneity tests were observed because they were required for the One Way ANOVA test with a confidence level of $\alpha = 95\%$ with three repetitions of the antibacterial activity test at each concentration. This data analysis aims to see a significant difference in each concentration of the preparation. H_0 is accepted. When the value of $\text{sig} > 0.05$, it means that there is no difference in the concentration of each extract. Meanwhile, if H_0 is rejected if the value of $\text{sig} < 0.05$ which means there is a significant difference, the difference that occurs in each extract concentration, a post hoc Tukey HSD test is carried out

RESULTS AND DISCUSSION

A. Antibacterial activity test results

Table 2nd. The results of the antibacterial activity test of the diffusion method based on the measurement of the diameter of the well (mm)

Test	Uji aktivitas antibakteri (mm)							
	<i>S.aureus</i>				<i>E.coli</i>			
	1	2	3	Rata-rata ± SD	1	2	3	Rata-rata ± SD
Cream Base (+ surfactant)	0,0	0,0	0,0	0,0 ± 0,0 ^a	0,0	0,0	0,0	0,0 ± 0,0 ^a
Cream Base (- surfactant)	0,0	0,0	0,0	0,0 ± 0,0 ^a	0,0	0,0	0,0	0,0 ± 0,0 ^a
Positive control (Sagestam® cream)	22,7	24,8	27,5	25,0 ± 2,0 ^b	15,0	14,0	13,8	14,3 ± 0,5 ^d
Telang leaf extract cream 10%	27,8	26,0	27,3	27,0 ± 0,8 ^b	16,3	17,0	18,5	17,3 ± 0,9 ^d
Telang leaf extract cream 20%	13,8	15,8	14,5	14,7 ± 0,8 ^c	13,0	14,0	15,5	14,2 ± 1,0 ^d

Information:

a: significant difference with cream base group (+ surfactant) ($p < 0.05$).

b: significant difference with cream base group (- surfactant) ($p < 0.05$).

c: significant difference with the positive group ($p < 0.05$).

The inhibition zone test was carried out to determine the presence of antibacterial activity in the extract of Telang leaves and the diameter of the inhibition zone against *S. aureus* and *E. coli* bacteria. In the cream base test group with the addition of surfactants there was no inhibition zone, this was due to the absence of nutritious ingredients that could cause the development and growth of bacteria and showed that the surfactant used in the cream base was not antibacterial. The same thing was also produced by the cream-based test group without the addition of surfactant, where there was no inhibition zone. The positive control group using Sagestam® cream containing 1% gentamicin sulfate had an inhibition zone indicating that the positive control had antibacterial activity against *S. aureus* and *E. coli* with the inhibitory zone activity shown to be greater against *S. aureus* than the inhibition zone that occurred against *E. coli*.

The 10% Telang leaf extract cream test group had an inhibitory zone comparable to the positive control group, where the inhibition zone was greater against *S. aureus* than the *E. coli* inhibition zone. This occurs because *S. aureus* which is a Gram positive bacterium contains thicker peptidoglycan than *E. coli* which is a Gram negative bacterium, where peptidoglycan is a polar layer found on the bacterial

cell wall, making it easier for the Telang leaf extract to penetrate the cell wall of *S. aureus*, than the cell wall of *E. coli* which contains a lot of lipopolysaccharide (LPS) which is non-polar which makes it more difficult for Telang leaf extract to penetrate the cell wall of *E. coli* bacteria (Collins & Lyne's, 2004; Rostinawati, 2009). The Telang leaf extract cream 20% also showed an inhibitory zone activity comparable to the positive control against *E. coli* but the Telang leaf extract cream 20% gave an inhibition zone that was not comparable to the positive control against *S. aureus*. This could be due to the ability of 20% Telang leaf extract to escape from the cream base to diffuse and affect the inhibition zone. Telang leaf extract cream 20% has a higher concentration than 10% Telang leaf extract, so it can inhibit the diffusion ability of Telang leaf extract to be separated from the cream base on the test medium.

B. Wound healing test results

Table 3^d. Results of measuring wound diameter

Test	Measuring wound diameters					
	Wound infections (<i>S.aureus</i> dan <i>E.coli</i>)			Wound without infections		
	7 th day	11 th day	14 th day	7 th day	11 th day	14 th day
Cream Base (+ surfactant)	4,9 ± 0,7 ^c	2,8 ± 0,6 ^a	1,8 ± 0,4 ^c	3,8 ± 0,4 ^c	2,4 ± 0,5 ^c	1,2 ± 0,4 ^c
Cream Base (- surfactant)	5,3 ± 0,5 ^a	3,2 ± 0,4 ^c	2,2 ± 0,4 ^a	4,1 ± 0,3 ^c	2,9 ± 0,6 ^c	1,6 ± 0,5 ^a
Positive control (Sagestam® cream)	3,3 ± 0,5 ^{ab}	1,9 ± 0,6 ^{ab}	0,8 ± 0,2 ^{ab}	3,1 ± 0,3 ^{ab}	1,3 ± 0,5 ^{ab}	0,6 ± 0,2 ^{ab}
Telang leaf extract cream 10%	3,7 ± 0,5 ^{ab}	1,9 ± 0,3 ^{ab}	0,9 ± 0,2 ^{ab}	3,1 ± 0,3 ^{ab}	1,5 ± 0,5 ^{ab}	0,8 ± 0,2 ^{ab}
Telang leaf extract cream 20%	3,9 ± 0,3 ^c	2,2 ± 0,4 ^{bc}	1,1 ± 0,4 ^{ab}	3,3 ± 0,5 ^{ab}	1,8 ± 0,4 ^{ab}	0,9 ± 0,2 ^{bc}

Information:

a: significant difference with the normal/without hyperglycemia group (p<0.05).

b: significant difference with the negative group (p<0.05).

c: significant difference with the positive group (p<0.05).

In the test group without hyperglycemia on day 7, the size of the wound closure diameter was still relatively small, especially in the treatment of wounds with infection so that the wound diameter was still quite wide. In the negative control group, which was only given a cream base without efficacious ingredients, the infection wound was widened so that there was no wound closure. The existence of this infection will cause the wound healing process to slow down and even stop, so that the wound does not get smaller even though the wound base already looks red, moist and healthy, so that the wound can develop into a chronic wound so that the wound healing process does not progress (Ariani et al, 2013).). In the positive control test group on day 7, both the diameter of the infected and non-infected wounds began to decrease so that they had a significant difference to the normal control group, the negative control group.

In the test group, the administration of 10% Telang leaf extract cream on infected and non-infected wounds showed comparable activity to the positive control. In the test group, administration of 20% Telang leaf extract cream on infected wounds on the 7th and 11th days showed activity that was not comparable to the positive control but on the 14th day the activity was comparable to that of the positive control. Different things were shown in the test group giving Telang leaf extract cream 20% on non-infected wounds which showed activity that was not comparable to the positive control. This is influenced by the difference in water volume between the 20% extract test group and the positive control where in the 20% Telang leaf extract test group there is an increase in water volume which causes a change in the balance between the oil phase and the water phase, resulting in a decrease in surface tension (Yumas M, 2016). Emulsifier in cream preparations has polar and non-polar groups in one molecule so that it binds non-polar oil and on the other hand, binds polar water so that there is a balance between the oil phase and the water phase. The addition of extracts with different levels can change the consistency of the cream preparation where the greater the level of extract added, the more concentrated

the consistency of the cream preparation which will affect the decrease in the spreadability of the cream preparation. (Widyaningrum, *et al.*, 2016).

CONCLUSION

1. Telang leaf extract cream with a concentration of 10% has antibacterial activity against *S. aureus* and *E.coli* by the diffusion method based on the diameter of the inhibition zone, namely the diameter of the inhibition zone against *S. aureus* 27.3 mm and *E. coli* 18.5 mm.
2. Telang leaf extract cream with a concentration of 10% has wound healing activity for *S.aureus* and *E.coli* infections based on wound diameter measurements on day 14, namely wound healing activity for infected wounds 0.9mm and non-infected wounds 0.8mm

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