Phytochemical Test and Effectiveness of Binahong Extract as Antibacterial and its Potential on Full Thickness Wound Diameter in Rattus Norvegicus

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Abstract: Binahong is a plant that contains active compounds and has potential as an anti-oxidant, anti-inflammatory, and anti-bacterial. The active compounds of binahong plants have been widely studied to play a role in the phases of the wound healing process. This study examined the phytochemicals, the potential of the extract as an antibacterial, and the effectiveness of binahong (*Anredera cordifolia*) gel on the diameter of full-thickness wounds in *Rattus norvegicus* rats. This type of research is in vitro and in vivo, using a true experimental post- test control design. Bacteria used in the study, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were tested using concentrations of binahong extract at 25%, 50%, 75%, and 100%. The research sample was *Rattus norvegicus* rats, which were divided into 5 treatment groups: negative control, positive control, and binahong gel doses of 10%, 20%, and 30%. The parameters used for the antibacterial test were the zone of inhibition and wound healing parameters seen from the measurement of wound diameter carried out on day 14. Statistical tests used descriptive analysis and one-way ANOVA test. The phytochemical screening test of binahong (*Anredera cordifolia*) extract contains alkaloid, flavonoid, triterpenoid, steroid, saponin, and tannin compounds. Binahong extract effectively inhibits *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria. There is a clear zone, which is the zone of inhibition. The most effective concentration of binahong extract in inhibiting Pseudomonas aeruginosa bacteria was obtained at a concentration of 75% and *Staphylococcus aureus* at a concentration of 25%. The results of the one-way anova test showed that there was a significant difference in the mean wound diameter between treatment groups ($p<0.05$). Games Howell's post hoc test found that the dose of binahong gel that is effective in wound healing is found in the 10% dose of binahong gel. Binahong contains active compounds of alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins that have the potential to be antibacterial and proven in wound healing, as seen from the decrease in wound diameter.

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1 INTRODUCTION

Physiopathological wound healing is a continuous and complex process that involves many interconnected factors (Liu et al., 2022). The wound healing process consists of interactions between cells, mediators, growth factors, and cytokines. Wound healing begins with blood clotting and the recruitment of inflammatory cells, which then continues to the proliferation phase. In the proliferative phase, keratinocytes will close the wound and form a new epithelial layer, fibroblasts will produce collagen matrix, and angiogenesis will occur (Fang et al., 2010; Patel et al., 2019). The state of open wounds will also become an area of bacterial colonization, which further develops into bacterial invasion and infection (Hutagalung et al., 2019). The bacteria identified in diabetic foot wounds are mostly gram- positive, namely *Staphylococcus aureus*, and gram- negative, namely *Pseudomonas aeruginosa* bacteria. Both Staphylococcus and Pseudomonas bacteria slow recovery, but Pseudomonas infection has a poorer prognosis (Suparwati et al., 2022). Gram-positive bacteria were obtained at 62.5%, and *Staphylococcus aureus* was the most dominant microorganism. Gram- negative organisms obtained 37.5%, and *Escherichia coli* is the bacterium that dominates gram-negative organisms (Rizqiyah et al., 2020).

Research on wound care explains that modern methods of wound dressing are more effective than conventional methods (Harish Rao, 2012; Nontji et al., 2015). A meta-analysis study on the comparison of modern wound dressing and conventional means that the principle of conventional methods is the same as that of modern methods, except that the normal use of saline and gauze dries faster so that it cannot maintain moisture on the wound surface (Kusumastuty & Dewi, 2020; Luh Titi Handayani, 2016). Modern wound dressing in the form of hydrocolloid film is waterproof, not permeable to infection, provides thermal insulation, is occlusive so as to provide a moist healing environment, encourages autolysis, and causes a decrease in local pH that allows the body's defense mechanisms to function effectively (Hidayat et al., 2021).

One of them is with the use of traditional medicine such as binahong leaves (*Anredera cordifolia* (Ten.) Steenis), which is a plant found in many yards and has antibacterial, antidiabetic, antiulcer, and anti- inflammatory properties (Anggraeni et al., 2018). Results of clinical studies and experiments Flavonoids can improve vascularity and decrease edema. Research has proven that flavonoids have anti-inflammatory effects. The therapeutic effect of the active compounds of medicinal plants has been widely studied, which plays a role in the phases of the healing process of diabetic ulcers (Refiani et al., 2021). The cost of treatment using modern dressings is more expensive compared to conventional wound care, conventional wound care with the addition of complementary therapies from traditional medicine is expected to accelerate the healing of diabetic ulcers

Alternative medicine is currently being developed as a comprehensive therapy, and an antioxidant. The content of flavonoids is also believed to have benefits in the wound healing process (Hasanoglu et al., 2001; Rohani, 2021). This plant also has flavonoid compounds that have anti-inflammatory effects, while saponins work as antiseptics that can stop or prevent the growth of microorganisms in wounds to avoid infection, increase the number of fibroblast cells, and stimulate collagen formation (Nury et al., 2019). Previous research revealed that binahong has active components such as flavonoids, saponins, and tannins that have anti-oxidant, anti-inflammatory, and anti-bacterial effects. It is also reported that binahong accelerates the wound healing process by increasing substances needed in the tissue regeneration process and proliferation phase such as tannins, saponins, triterpenoids, alkaloids, ascorbic acid, and steroids (Anggraeni et al., 2018; Kintoko &; Desmayanti, 2016). The results of examination using UV-Vis and FT-IR spectra methods, flavonoid compounds identified in ethyl acetate extract of binahong leaves are flavonol groups, while in ethanol extracts flavone groups are identified (Nisa et al., 2012).

2 METHODS

The study was included in the Laboratory Experimental study with a complete randomized design (RAL). This research design uses pre- and post-test control design. Animal House Laboratory, Faculty of Medicine, Unsri. The study sample was male Wistar, with a total sample of 25 in each group of 4 samples. The treatment group consisted of a negative control, a positive control, 10% gel, 20% gel, and 30% gel. The parameters of the study were the inhibitory zone of bacteria and the diameter of the wound. The diameter of the initial wound is ±15 cm. Treatment lasts 14 days; the gel is given twice a day (morning and evening). Descriptive and inferential data analysis using one-way anova test followed by post hoc test games howell.

3 RESULTS

3.1 Qualitative Phytochemistry

Binahong extract is obtained by maceration or soaking of binahong dry simplisia. Simplisia is blended until smooth, then soaked in 96% ethanol and replaced with a total turnover of 2 times with a soaking time of 3x24 hours. The results of soaking are then dried in the oven for 7x24 hours at a temperature of 36–37 °C until the moisture content and solvent content in the maceration results are lost and leave a thick extract from binahong. A total of 850 grams of binahong dry simplisia blended were dissolved in 4 liters of 96% ethanol and obtained 28.37 grams of binahong extract.

Tabel 1. Qualitative Phytochemical Test Results

No.	Test Type	How it Works	Result	
1.	Alkaloid: Dragendro ff	Extract + 5 mL HCL 2%, stir until homogeneo us then divide into 3 tubes. Tube 1 + 3 drops of dragendroff reagent	Red- orange deposits	+
	Alkaloid: Mayer	Tube 2+3 drops of mayer reagent	Yellowish sediment	+
	Alkaloid: Wagner	Tube 3 + wagner reagent	Chocola te sediment	+
2.	Flavonoid	Extract + methanol + 0,5gram metal magnesium + 5 drops of HCL concentrate lau heated	Red- orange deposits	+
3.	Triterpenoid	Extract + 0.5 ml chloroform + 0.54 ml of anhydrous acetic acid + 2 ml of concentrated sulfuric acid through the wall	Formed violet rings	+
4.	Steroid	Extract + 0.5 ml chloroform + 0.54 ml of anhydrous acetic acid + 2 ml of concentrated sulfuric acid through the wall	Formed bluish- green color	+
5.	Saponin	Extract + 10 ml of hot water, cool and then shake vigorously for 10 seconds	Froth formed 1.5 cm high for 10 minutes	+
6.	Tannin	Extract + 2ml FeCL2 1%	Blackish blue	+

3.2 Binahong Extract as antibacterial *Pseudomonas aeruginosa* and *Staphylococcus aureus*

The antibacterial test results of binahong extract against *Pseudomonas aeruginosa* bacteria and *Staphylococcus aureus* bacteria were seen in the inhibitory zone formed after the test shown in the figure 1:

A B

Figure 1: Antibacterial Test Results of Binahong Extract against *Pseudomonas aeruginosa and Staphylococcus aureus* bacteria clear zone which is an inhibitory zone (A) *Pseudomonas aeruginosa* bacteria (B) *Staphylococcus aureus* bacteria

The results of the descriptive analysis of the inhibitory zones formed are presented in the figure 2 and figure 3.

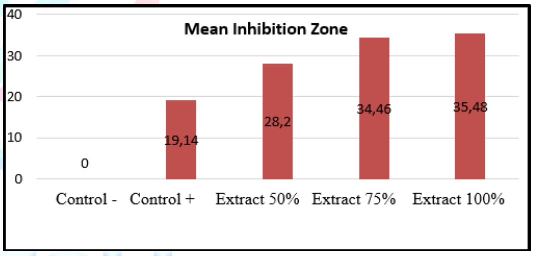

Figure 2: Graph of the Mean Inhibition Zone *Pseudomonas aeruginosa*

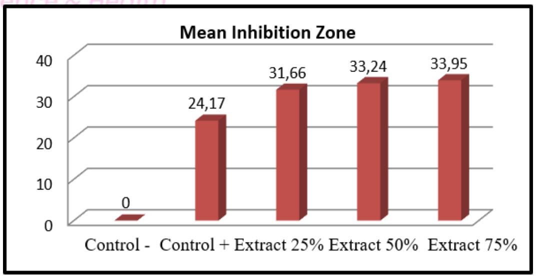

Figure 3: Graph of the Mean Inhibition Zone *Staphylococcus aureus*

The inhibitory activity of binahong ethanol extract against *Pseudomonas aeruginosa* and

Staphylococcus aureus bacteria by the Kirby-Bauer method showed positive results marked by the formation of an inhibitory zone around the test disc. In figure 2. Showing from all treatment groups, binahong extract concentration of 100% has the largest mean inhibitory area of 35.48 mm, while the smallest mean inhibitory area is found at a concentration of 50% (28.20 mm). The negative control group showed no inhibitory effect on *Pseudomonas aeruginosa* bacteria, and the positive control group showed inhibition with an average of 19.14 mm. Thus, the higher the concentration of binahong ethanol extract tested, the greater the inhibitory zone of *Pseudomonas aeruginosa* bacteria formed.

From all treatment groups, binahong extract concentrations of 75 percent have the largest mean inhibition area of 33.95 mm, while the smallest mean inhibitory area is 25% concentration (31.66 mm). Treatment 1 (negative control) showed no inhibitory effect on *Staphylococcus aureus* bacteria and treatment 2 (positive control) showed inhibition with an average of 24.17 mm.

3.3 Preliminary wound diameter measurement

The results of measuring the diameter of the wound at the beginning of day 0 showed no real difference in the average diameter of the wound between treatment groups, so it was continued with the provision of intervention in each treatment group. The results of measuring the diameter of the 0th day wound was presented in the table 2.

Tabel 2. Day 0 Wound Diameter Homogeneity Test

Group	N	Average ± SD	p
Negative control	4	15.91 ± 0.07	0,086
Positive control	4	15.43 ± 0.02	
Gel binahong 10%	4	15.40 ± 0.14	
Gel binahong 20%	4	15.56 ± 0.28	
Gel binahong 30%	4	15.45 ± 0.39	

levene test p>0.05

3.4 Wound Diameter Day 3, 7 day and 14 day

Results of wound diameter analysis day 3, day 7 and day 14 in each treatment group. Descriptively the wound diameter from day 3 to day 7 after injury decreased the wound diameter in each group both in negative, positive, binahong gel doses of 10%, 20% and 30%. The full results are presented in the table 3.

3.5 Potension Binahong Gel against Wound Healing Day 14

The results of macroscopic early wound (pre) and post-day 14 wound healing in each treatment group, where it can be seen that in the negative control group the wound area is still extensive and the wound healing process is still ongoing compared to the wound area that has been seen shrinking in picture (B), namely the administration of povidon iodine and in picture (C), (D), (E) namely the administration of binahong gel doses of 10%, 20% and 30%.

Table 3: Diameter Wound of day 3, day 7 and day 14

Group	n	Day 3	Day 7	Day 14
		Average ± sd	Average ± sd	Average ± sd
Negative control	4	15.53 ± 0.26	15.05 ± 0.16	9.95 ± 0.85
Positive control	4	15.37 ± 0.63	11.65 ± 1.91	4.45 ± 0.02
Gel binahong dosis 10%	4	14.17 ± 0.56	10.33 ± 1.05	3.56 ± 0.80
Gel binahong dosis 20%	4	14.17 ± 0.30	10.48 ± 0.20	5.53 ± 0.09
Gel binahong dosis 30%	4	15.07 ± 0.46	13.19 ± 0.56	7.4 ± 0.30

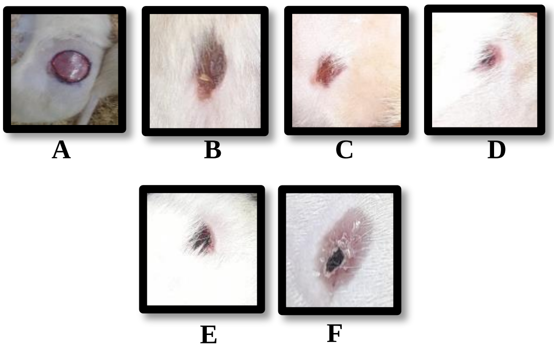

Figure 1: Day 14 Wound Healing (A)Initial wound diameter; (B)Negative control; (C)Positive control; (D)10% binahong gel; (E)20% binahong gel; (F)30% binahong gel.

The results of the analysis of wound diameter comparison between treatment groups, the average negative control was higher than that of the positive control group, binahong gel doses of 10%, 20% and 30%. Statistical tests found that there was a significant difference in the average diameter of wounds between groups (p < 0.05). The full analysis results are presented in the table 4:

Table 4: Binahong Gel's Potential for 14th Day Wound Healing

Group	N	Day 14	p
		Average±sd	
Negative control	4	9.95 ± 0.85	
Positive control	4	4.45 ± 0.02	
Gel binahong 10%	4	3.56 ± 0.80	<0,0001
Gel binahong 20%	4	5.53 ± 0.09	
Gel binahong 30%	4	7.4 ± 0.30	

one way anova test (p<0.0001)

The comparison of wound diameter in each binahong gel treatment dose of 10%, 20% and 30% and positive control was significantly different from negative control while binahong gel dose was 10% more effective in wound healing and did not differ markedly from positive control compared to binahong gel dose of 20% and 30%.

Table 5: *Post hoc test Games Howell*

Group	Control (-)	Control (+)	Gel 10%	Gel 20%	Gel 30%
Negative Control		0,024	0,003	0,035	0,084
Positive Control			0,500*	0,006	0,010
Gel binahong 10%	0,024	0,500		0,147	0,017
Gel binahong 20%	0,003	0,006	0,147		0,027
Gel binahong 30%	0,084	0,010	0,017	0,027	

4 DISCUSSION

The results of the analysis of the comparison of wound diameters between treatment groups, it was found that there was a significant difference in the average wound diameter between groups (p < 0.05). The comparison of wound diameter in each binahong gel treatment dose of 10%, 20% and 30% and positive control was significantly different from negative control while binahong gel dose was 10% more effective in wound healing and did not differ markedly from positive control compared to binahong gel dose of 20% and 30%. In line with the results of research by Nisa Hanifah, et al (2019**)** showed binahong extract gel (*Anredera cordifolia*) at concentrations of 2.5% and 5% effective in reducing the diameter of wound contractions in RAS patients. The most effective concentration in wound healing in RAS patients is a concentration of 2.5% (Nisa Hanifah, Anindiya Kartika Sari, Adhika Ilham Gemilang, 2019).

Binahong plants can accelerate the wound healing process by increasing substances needed in the process of tissue regeneration and proliferation phases such as saponins, alkaloids, tannins, steroids, triterpenoids, flavonoids, ascorbic acid (Esimone CO, Ibezim EC, 2006; Kintoko, 2015). Flavonoids are a class of compounds with various biological functions, becoming an important source of new products with pharmaceutical potential, including the treatment of skin wounds (Carvalho et al., 2021). Binahong extract with a concentration of **35%** also showed faster healing results in diabetic mouse incision wounds compared to placebo gel (control), binahong leaf extract concentrations of 25% and 30%, which were seen from histopathological microscopic images. The densest collagen density is found in binahong leaf extract gel concentration of 35%. Giving binahong leaf extract gel can increase collagen density and accelerate the healing process of incision wounds in mice with complications of diabetes where concentrations are 35% more effective than concentrations of 30% and 25% (Sihotang et al., 2019).

The metabolites present in Binahong leaves are responsible for the pharmacological effects they perform. These chemicals contribute to the anti-inflammatory, antimicrobial, wound healing, antihypertensive, andanti-perlipidemia properties in Binahong leaves (Salim et al., 2021). Increasing the average granulation tissue thickness and better healing time there was an experimental group given binahong leaf extract with a concentration of 40% where binahong leaf extract 40% has the potential to accelerate the increase in granulation tissue thickness and burn healing time better than extract and binahong concentration of 20% (Betriksia et al., 2018).

5 CONCLUSIONS

Based on the results of the study and the objectives of this study, it can be concluded that:

1) The results of phytochemical screening test of binahong extract (*Anredera cordifolia*) contain alkaloid compounds, flavonoids, triterpenoids, steroids, saponins and tannins.
2) Binahong extract (*Anredera cordifolia*) is proven.as an anti-bacterial Pseudomonas aeruginosa. The inhibitory zones of binahong ethanol extract concentrations of 50%, 75%, and 100% formed were 28.20±1.59 mm (very strong), 34.46±1.14 mm (very strong), 35.48±1.38 mm (very strong). The inhibitory zone of positive control is 19.14 ± 0.56 mm (strong).
3) Binahong extract (*Anredera cordifolia*) is proven to be anti-bacterial Staphylococcus aureus. The largest 75% concentration inhibition zone is 33.95 mm, while the smallest inhibition is 25% concentration (31.66 mm). The negative control showed no inhibitory effect on bacteria and the positive control showed inhibition with an average of 24.17 mm.
4) Binahong gel (*Anredera cordifolia*) has the potential to heal wounds in non-diabetic mouse models proven to decrease wound diameter between treatment groups.
5) The dose of binahong gel (*Anredera cordifolia*) that is effective in wound healing in non-diabetic mouse models is found in binahong gel dose 10%.

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