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Original Research Paper

Effect of *Bandotan (Ageratum conyzoides Linn)* leaf extract and wild honey provision on cut wound healing in mice

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Abstract
Cuts are a common form of injury caused by trauma from sharp objects such as knives, razor blades, or other sharp objects. In Indonesia, traditional wound treatment is still widely used, one of which is by using *Bandotan* leaves (*Ageratum conyzoides Linn*), which is known to have the ability to stop bleeding and accelerate wound healing. In addition, wild honey from *Apis dorsata* bees also contains various active compounds that can be significant for the wound healing process. Data from *Riskesdas* (*Riset Kesehatan Dasar* - Basic Health Research) showed that the prevalence of cut/slash/stab wound cases in South Sulawesi in 2018 reached 27.76%, so an effective and natural alternative treatment is needed. This study aims to determine the content of phytochemical compounds in *Bandotan* leaf extract and wild honey; and compare their effectiveness on cut healing in mice (*Mus musculus*), both macroscopically and histologically. This study employed a quantitative experimental method with a Pretest-Posttest Control Group Design. The results show that *Bandotan* leaf extract contains alkaloids, flavonoids, steroids, and saponins; while wild honey contains flavonoids and saponins. Macroscopically, wild honey is more effective in accelerating wound healing than the other groups. However, microscopically, the *Bandotan* leaf group shows higher epidermal thickness. Complete re-epithelialization occurs in all groups, while keratinization does not only occur in the negative control group. In conclusion, wild honey is more clinically effective in accelerating wound healing, while *Bandotan* leaves show potential for tissue regeneration based on histology.

Keywords: *Bandotan* Leaf Extract; Cut; Wild Honey; Mice (*Mus musculus*)

1. Introduction

Skin as a protective layer of the body has vital functions, one of which is to maintain tissue integrity against physical, chemical, and infectious trauma (Azis et al., 2023; Irenesia et al., 2023). Cuts are a common form of injury caused by sharp objects such as knives or razor blades, which cause bleeding, sympathetic stress, and the risk of infection (Meliawati et al., 2022). In Indonesia, the prevalence of the wounds is still high, especially in South Sulawesi that reached 27.76% in 2018 (RiskesdasTeam, 2018). This shows the importance of effective and accessible wound healing therapies to the community.

The use of herbal medicine is increasingly preferable because it is safer and more economical. *Bandotan* leaves (*Ageratum conyzoides Linn*) are known to contain flavonoids, saponins, and alkaloids that have anti-inflammatory effects and accelerate tissue regeneration (Hutauruk et al., 2022; Natali, 2023). Similarly, wild honey from *Apis dorsata* bees has been confirmed to accelerate wound healing through stimulation of fibroblasts and epithelialization (DwiUtami et al., 2023; Syafitri et al., 2022).

However, there have not been many studies that compare the two directly in one experimental design. This study was conducted to compare the effectiveness of *Bandotan* leaf extract and wild honey on cut healing in mice, both macroscopically and microscopically.

2. Research Methods

This study used a quantitative experimental design of Pretest-Posttest Control Group Design to evaluate the effect of *Bandotan* leaf extract (*Ageratum conyzoides Linn*) and wild honey (*Apis dorsata*) on cut wound healing in male mice aged 3-4 months. A total of 32 mice were randomly selected and divided into four groups: negative control (no treatment), positive control (Povidone iodine 10%), *Bandotan* leaf extract treatment, and wild honey treatment. A 2 cm cut wound was made on day 6, then the intervention was given from day 8 to day 16. Macroscopic observations were made on days 0, 3, 6, and 9 to assess wound contraction. Meanwhile, microscopic observations were made after euthanation with parameters of reepithelialization, epidermal thickness, keratinization, and remodeling. Tissue staining performed by using the Hematoxylin-Eosin (HE) method. The tools and materials included: cages, food and drink containers, digital scales, rulers, cotton buds, scalpels, shavers, masks, stirring rods, beaker glasses, drip pipettes, measuring cups, handscoen, blenders, *hypafix*, preparations, microscopes, and observation sheets. Test materials consisted of *Bandotan* leaves, *Apis dorsata* wild honey, 96% ethanol, povidone iodine, and distilled water. Data were analyzed by using SPSS. Wilcoxon test was employed for macroscopic data that were not normally distributed, followed by Kruskal-Wallis and Mann-Whitney tests for comparison between groups. Histological analysis was performed by one-way ANOVA and Tukey test, as well as Fisher Exact test for tissue remodeling.

3. Results and Discussion

3.1.Phytochemical Screening

Tabel 1. Phytochemical Screening of Bandotan Leaf Extract and Wild Honey

Compound Content	*Bandotan* Leaves	Wild Honey
Alkaloid	+	-
Flavonoid	+	+
Steroid	+	-
Fenolik	-	-
Saponin	+	+

Source: Primary Data, 2024

Based on the phytochemical screening results in Table 1, *Bandotan* Leaf extract contains alkaloid, flavonoid, steroid, and saponin compounds. Meanwhile, the phytochemical screening results of Wild Honey exposed the presence of flavonoid and saponin compounds.

3.2.Effect of *Bandotan* Leaf Extract and Wild Honey on Cut Length

Table 2. Pre-Test and Post-Test Difference Table

	Pre-Test		Post-Test		
Group	Mean	SD	Mean	SD	p-value
Negative Control	2	0	0.15	0.23	0.024
Positive Control	2	0	0.07	0.16	0.020
Bandotan Leaves	2	0	0.92	0.13	0.024
Wild Honey	2	0	0.17	0.19	0.026

Source: Primary Data (2024)

Notes:

Wilcoxon test has a significant difference if the *p*-value (<0.05).

Table 2 shows the results of statistical analysis to assess the effectiveness of all treatment groups in cut healing through comparison of pre-test and post-test results. In the negative control group, the *p*-value of 0.024 showed a significant difference between the pre-test and post-test, which means that the treatment in this group is effective in accelerating cut healing. The positive group showed a *p*- value of 0.020 also indicating a significant difference and effectiveness in healing the cut. In the *Bandotan* Leaf group, the *p*-value of 0.024 showed a significant difference between the pre-test and post-test results, confirming that *Bandotan* Leaf significantly contributed to the healing of the cut wound. Finally, the Wild Honey-treated group was also significantly effective in accelerating cut healing. Overall, the *p-values* showed results smaller than 0.005 confirming that each treatment administered in this study had a positive and significant impact on the cut healing process.

Table 3. Table of Post-Test Differences of All Groups

Group	Mean (cm)	*p*-value
Negative	0.150	
Positive	0.067	0.002
Bandotan Leaves	0.917	
Wild Honey	0.167	

Source: Primary Data (2024)
Notes:
Kruskal Wallis test has a significant difference if p-value (<0.05)

Table 4. Table of Specific Differences of Post-Test between Groups

Group (i)	Group (ii)	p-value
a	b	0,461
	c	0,003
	d	0,929
b	c	0,002
	d	0,338
c	d	0,003

Notes:
a = Negative Control
b = Positive Control
c = *Bandotan* Leaves
d = Wild Honey
Mann-Whitney test has a significant difference if *p-value* (<0.05) Group (i) and (ii): Comparison Group.

Based on Table 3, the comparison of all groups shows a significance value of 0.002 ($p < 0.05$), meaning that there are significant differences between treatment groups. Table 4.4 shows the results of statistical analysis to specifically assess the difference in effectiveness between groups. The negative control group and the positive control group showed no significant difference with a *p-value* of 0.461. The negative control group and the *Bandotan* Leaves treatment group showed a significant difference characterized by a *p-value* of 0.003, which means that the negative control group and the *Bandotan* Leaves treatment group were effective in accelerating cut healing.

Meanwhile, the negative control group and the Wild Honey treatment group had no significant difference with a *p-value* of 0.929. The positive control group and the *Bandotan* Leaves treatment group showed a significant difference with a *p-value* of 0.002 confirming that the positive control group and

the *Bandotan* Leaves treatment group contributed significantly to the healing of the cut wounds. The positive control group and the Wild Honey treatment group showed no significant difference with a *p-value* of 0.338. Finally, the *Bandotan* Leaves and Wild Honey treatment groups had a *p-value* of 0.003, indicating that the *Bandotan* Leaves and Wild Honey treatment groups were also significantly effective in accelerating cut healing.

3.3.Observation of Cut Length and Cut Healing Time

Observation of the length of the cut wound in mice (*Mus musculus*) was carried out on days 0, 3, 6, and 9. On day 0, all mice (*Mus musculus*) had an cut wound length of 2 cm before treatment. The results of cut wound measurements in mice (*Mus musculus*) are presented in the following table.

Table 5. Average Length of Cut Wound

Group	Average leght of Cut Wound (cm)			
	H-0	H-3	H-6	H-9
Negative Control	2	1.17	0.37	0.15
Positive Control	2	1.13	0.22	0.07
Bandotan Leaves	2	1.72	1.3	0.92
Wild Honey	2	1.2	0.28	0.17

Source: Primary Data (2024)

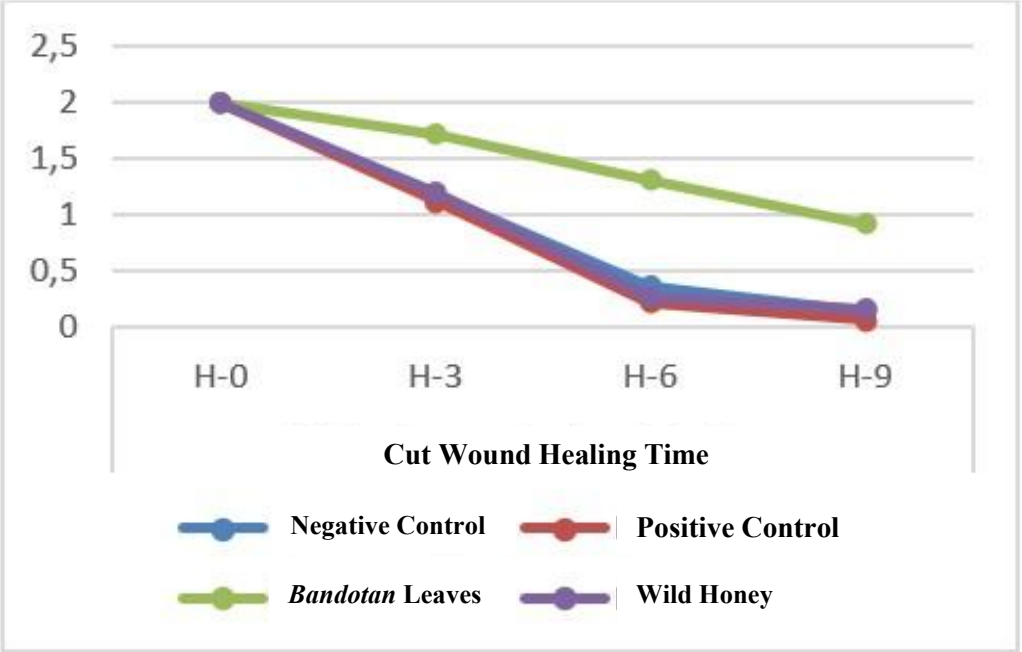

Figure 1. Graph of Change in Average Length of Cut Wound
Source: Primary Data, 2024

Table 6. Comparison of Cut Wound Healing Effectiveness on Day 3

Day	Group (i)	Group (ii)	*p*-value	*p*-value Bonferroni
	a	b	0.868	5.208
		c	0.004	0.024
		d	0.738	4.428
3	b	c	0.003	0.018
		d	0.669	4.014

Day	Group (i)	Group (ii)	*p*-value	*p*-value Bonferroni
	c	d	0.005	0.03

Notes:
a = Negative Control
b = Positive Control
c = *Bandotan* Leaf
d = Wild Honey
Mann-Whitney test has a significant difference if *p*-value (<0.05)
Group (i) and (ii): Comparison Group

It can be concluded that on day 3, there was a significant decrease in cut wound length in the positive control group with an average wound length of 1.13 cm. This is supported by the value of *p*-value when compared to *Bandotan* Leaves and negative control (0.018 and 5.208). Wild Honey showed an average wound length of 1.2 cm, slightly better than the negative control, but the *p*-value when compared to the positive control was still higher (4.428 and 4.014). The results showed that there was a significant difference between the Wild Honey group and the positive control. This indicates that the positive control group began to show its effectiveness in accelerating cut wound healing and was faster than the other groups on day 3.

Table 7. Comparison of Cut Wound Healing Effectiveness on Day 6

Day	Group (i)	Group (ii)	p-value	p-value Bonferroni
	a	b	0,803	4,818
		c	0,003	0,018
		d	0,007	0,042
6	b	c	0,003	0,018
		d	0,004	0,024
	c	d	0,004	0,024

Notes:
a = Negative Control
b = Positive Control
c = *Bandotan* Leaves
d = Wild Honey
Mann-Whitney test has a significant difference if the *p*-value (<0.05) Group (i) and (ii): Comparison Group

It can be concluded that on day 6, there was a significant decrease in cut wound length in the positive control group with an average wound length of 0.22 cm. This is supported by the value of *p*-value when compared to *Bandotan* Leaves and negative control (0.018 and 4.818). Wild Honey showed an average cut wound length of 0.28 cm, slightly better than the negative control, but the *p-value* showed higher results when compared to the positive control (0.042 and 0.024). The results showed that there was a significant difference between the Wild Honey group and the positive control. This indicates that the positive control group began to show its effectiveness in accelerating cut wound healing and was faster than the other groups on day 6.

Table 8. Comparison of Cut Wound Healing Effectiveness on Day 9

Day	Group (i)	Group (ii)	p-value	p-value Bonferroni
	a	b	0,461	2,766
		c	0,003	0,018
		d	0,655	3,93
9	b	c	0,002	0,012

Day	Group (i)	Group (ii)	p-value	p-value Bonferroni
		d	0,338	2,028
	c	d	0,003	0,018

Notes:
a = Negative Control
b = Positive Control
c = *Bandotan* Leaves
d = Wild Honey
Mann-Whitney test has a significant difference if *p*-value (<0.05) Group (i) and (ii): Comparison Group

It can be concluded that on day 9, there was a significant decrease in cut wound length in the positive control group with an average cut wound length of 0.07 cm. This is supported by the value of *p-value* when compared to *Bandotan* Leaf and negative control (0.012 and 2.766). Wild Honey showed an average cut wound length of 0.17 cm, but was still better than the negative control (0.15 cm), there was a difference in *p*-value when compared to the positive control which was still higher (2.766 and 0.012). The results showed that there was a significant difference between the Wild Honey group and the positive control. This indicates that the positive control group began to show its effectiveness in accelerating cut wound healing and was faster than the other groups on day 9. Although the positive control showed better effectiveness, the Wild Honey group also had almost equal effectiveness.

3.4.Microscopic Results of Cut Wound Healing

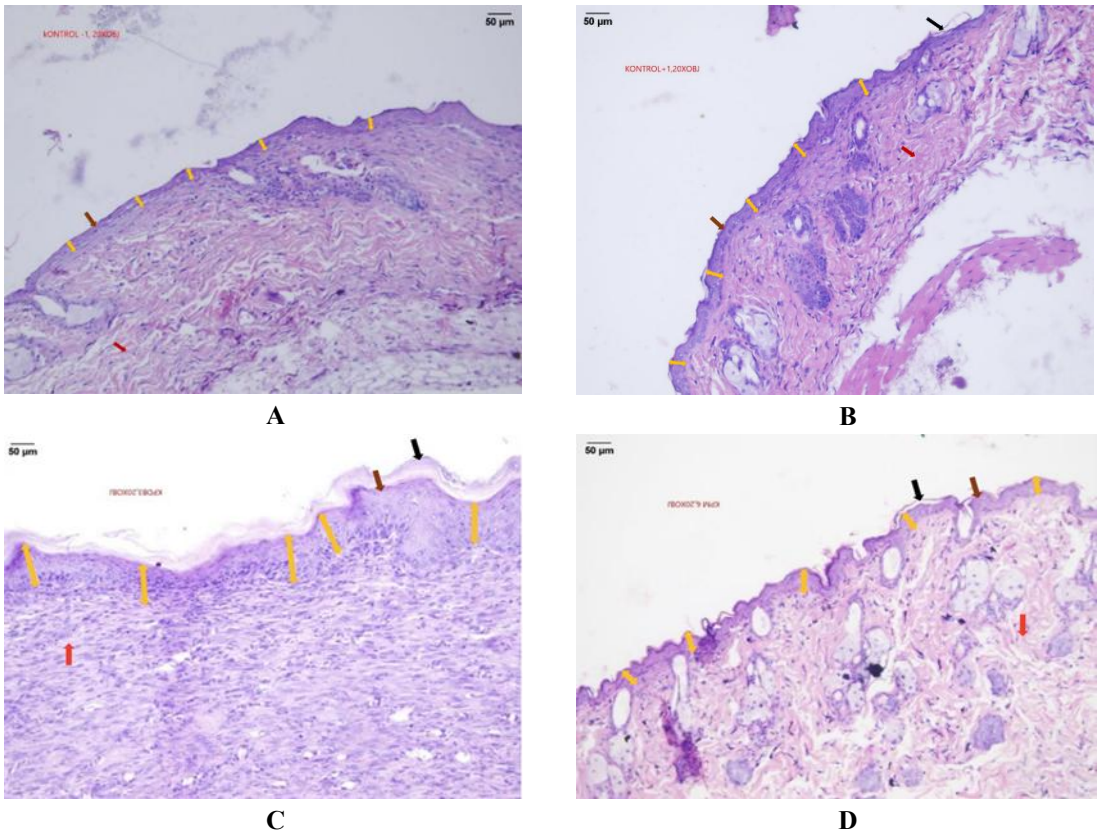

Figure 2. Microscopy of Mouse Skin (20 x Magnification)
Source: Primary Data, 2024

Negative Control Group (A), Positive Control Group (B), *Bandotan* Leaves Treatment Group (C), Wild

Honey Treatment Group (D), Reepithelialization (Brown Arrows), Remodeling (Red Arrows), Keratinization (Black Arrows), Epidermal Thickness (Orange Arrows)

Table 9. Epidermal Thickness

Group	Epidermal Thickness (µm)
Control (-)	52.67±24.41[a]
Control (+)	62.67±31.08[a]
Bandotan Leaves Treatment Group	171.83±50.55[b]
Wild Honey Treatment Group	73.83±33.87[a]

Source: Primary Data (2024)

Notes:

One Way *ANOVA* test

Different superscripts (^{ab}) in each same column indicate significant differences ($p<0.05$).

Microscopic examination results obtained in the negative control group (Figure A), positive control group (Figure B), *Bandotan* Leaves treatment group (Figure C), and Wild Honey treatment group (Figure D) showed intact reepithelialization (Brown Arrow). The negative control group (Figure A), the positive control group (Figure B), and the Wild Honey treatment group (Figure D) showed collagen fibers that were arranged regularly; so that, it could be said to be complete remodeling (Red Arrows). Meanwhile, in the *Bandotan* Leaves treatment group, the collagen fibers were not so visible; so that, it could be said to be partial remodeling (Red Arrows). Further, the positive control group (Figure B), *Bandotan* Leaves treatment group (Figure C), and Wild Honey treatment group (Figure D) showed keratinization (Black Arrow), while the negative control group did not show any keratinization.

Based on Table 9, the results of the One-way *ANOVA* test showed a significant difference ($p <0.05$) in the thickness of the epidermis (Orange Arrow) microscopically. In this study, the results of epidermal thickness presented in sequential from the lowest to the highest starting from the negative control group (52.67 µm), positive control group (62.67 µm), Wild Honey treatment group (73.83 µm), and *Bandotan* Leaves treatment group (171.83 µm) showed significant differences in each group. Based on the *Tukey* Test result, the superscript notation shows a significant difference. The negative control group, positive control group and Wild Honey treatment group did not show a significant difference but showed a significant difference to the *Bandotan* Leaves treatment group.

Table 10. Frequency Distribution Test

Group	Complete	%	Partial	%
Negative Control	2	33	4	67
Positive Control	4	67	2	33
Bandotan Leaves Treatment	1	17	5	83
Wild Honey Treatment	3	50	3	50

Source: Primary Data (2024)

Notes:

Score: Complete= 2 (Collagen fibers are arranged regularly) Partial = 1 (Collagen fibers are not so visible)

Based on Table 10, the results of the Frequency Distribution Test show that there is no significant difference ($p>0.05$) on the criteria for complete and partial remodeling in microscopic results. The negative control group had 33% complete remodeling and 67% partial remodeling, the positive control group had 67% complete remodeling and 33% partial remodeling, the *Bandotan* Leaves treatment group had 17% complete remodeling and 83% partial remodeling. Finally, the Wild Honey treatment group had 50% complete remodeling and 50% partial remodeling.

Table 11. Specific Differences in Complete and Partial Remodeling Criteria between Groups

Group (i)	Group (ii)	p-value Fisher
a	b	0,567
	c	1,000
	d	1,000
b	c	1,000
	d	0,245
c	d	0,545

Source: Primary Data (2024)
Notes:
a = Negative Control
b = Positive Control
c = *Bandotan* Leaves
d = Wild Honey
Fisher's exact test has a significant difference if p-value (<0.05) Group (i) and (ii): Comparison Group

Based on Table 11, the results of the Fisher's Exact Test show that there is no significant difference (p>0.05) in the results of specific comparison of complete and partial remodeling between groups on microscopic results. The negative control group compare to the positive control group, the Wild Honey treatment group and the *Bandotan* Leaves treatment group had no significant difference with *p*-values of 0.567, 1.000, and 1.000, respectively. The positive control group compare to the Wild Honey treatment group and the *Bandotan* Leaves treatment group had no significant difference with *p*-values of (1.000 and 0.245) respectively. Finally, the *Bandotan* Leaves treatment group and the Wild Honey treatment group had no significant difference with a *p*-value of 0.545.

3.5. Discussion

This study shows that Povidone iodine as the positive control provides the best effectiveness in cut wound healing, followed by wild honey and *Bandotan* leaves extract. The effectiveness of wild honey is shown to be statistically significant in accelerating wound contraction, approaching the effectiveness of Povidone iodine (Mustafa et al., 2020; DwiUtami et al., 2023). The flavonoids and saponins in wild honey are predicted to play a role in stimulating fibroblasts, anti-inflammation, and accelerating re-epithelialization (Syafitri et al., 2022). Meanwhile, *Bandotan* leaves containing alkaloids, flavonoids, and steroids have a positive effect on epidermal thickness, indicating regenerative potential even though macroscopic healing is slower (Fahrezi et al., 2021; Hutauruk et al., 2022).

The difference in effectiveness between the two ingredients is also influenced by the concentration and diffusion ability of the active compounds. The high concentration of *Bandotan* leaves extract may reduce the penetration of active substances into the skin tissue, leading to a slower healing process (Nurqadriasti et al., 2019). In addition, other factors such as animal stress, nutrient intake, and wound depth also influence the variation in results (Anjarwati et al., 2024; Aziz et al., 2022). The wound healing process involves tissue re-epithelialization and remodeling, which is supported by bioactive compounds such as flavonoids that stimulate the release of TGF-β and VEGF (Safani et al., 2019). Histological results showed that although honey was more clinically effective, *Bandotan* leaves showed promising regenerative activity. Further research is recommended to explore the combination of both ingredients as well as a more stable and homogeneous gel dosage formulation (Praseptyawati, 2021; Putri, 2023).

Limitations in this study include possible inconsistencies between the results obtained and the expected hypothesis. This may be due to the selection of inappropriate methods or the presence of poorly controlled external factors that affect the results that cannot be predicted in advance. It is expected that further research will be conducted to determine the levels of compounds contained in *Bandotan* Leaves extract and *Apis dorsata* Wild Honey.

4. Conclusion

This study shows that *Apis dorsata* wild honey is more effective macroscopically in accelerating cut wound healing in mice compared to *Bandotan* (*Ageratum conyzoides Linn*) leaves extract, negative control, and positive control. However, microscopically, *Bandotan* leaves shows the highest epidermal thickness and complete re-epithelialization, indicating the potential for tissue regeneration. Both materials shows good keratinization formation, although complete tissue remodeling is more dominant in the positive control group. Thus, wild honey has the potential to be used as a natural wound healing agent, while *Bandotan* leaves are promising in terms of histological structure.

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