Assessment of anti-inflammatory of stem extract of Musa balbisiana in wound healing on rats (Rattus norvegicus)

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Assessment of anti-inflammatory of stem extract of *Musa balbisiana* in wound healing on rats (*Rattus norvegicus*)

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Abstract

Background: *Balbisiana* is a plant that contains flavonoids, which are antioxidants with high anti-inflammatory activity. This antioxidant activity can help the wound-healing process.
Purpose: To determine the anti-inflammatory effect of *balbisiana* extract to help wound healing.
Method: The study used 15 rats, which were divided into five groups: a positive control group, a negative control group, a 10% *balbisiana* extract group, a 25% *balbisiana* extract group, and a 40% *balbisiana* extract group. Statistical analysis was performed using the Shapiro-Wilk test to test the normality of the distribution of continuous quantitative data. Levene's test was then used to assess the statistical homogeneity of variance between groups. Welch's One-Way ANOVA was used to analyze the data. Post-hoc analysis was performed using the Games-Howell test.
Results: The results of the study showed that *balbisiana* extract had significant anti-inflammatory activity, especially at a concentration of 40%. This was demonstrated by the results of the study, which showed that rats treated with 40% *balbisiana* extract had significantly improved wound healing compared to the control groups.
Conclusion: This study shows that *balbisiana* extract has anti-inflammatory activity in helping the wound healing process.

Keywords: Anti-Inflammatory; *Balbisiana*; Wound Healing.

INTRODUCTION

Injury is damage or loss of body tissue that arises due to disruption of the body's defense system. Variations in the shape of the wound can occur depending on the cause, some are open and some are closed. For example, an open wound can be an incision or incision wound, which is characterized by the presence of linear tears in the skin and the underlying layer of tissue (Ida, Noer, & Parenrengi, 2020). In the wound healing process, there are generally four consecutive stages, namely the hemostasis phase (0 to several hours after injury), the inflammatory phase (1-3 days), the proliferation phase (4-21 days), and the renovation phase (21 days to 1 year) (Landén, Li, & Ståhle, 2016). Some examples are wound treatment using 0.9% NaCl, which usually causes the inflammatory phase to last for 1-6 days (Apriliyasari, & Endro, 2013). One other method involves the application of moist dressing using tulle grass containing 1% framycetin sulfate in humid conditions. This approach was able to speed up the wound healing process, and an increase in the neutrophil profile showed progress in healing by day 12 (Erwin, Rusli, & Jones, 2020).

Wound management can involve the use of several plants that have been identified and processed into herbal extracts, for example, Kirinyuh leaf extract (*Chromolaena odorata*), which has been shown to accelerate wound healing (Amfotis, Suarni,

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& Arpiwi, 2022). There is also Binahong leaf extract (*Anredera cordifolia (Tenore) Steen*) which is believed to have properties including accelerating the recovery of surgical wounds, healing various internal wounds, and external wounds, strengthening endurance, and much more (Erwin, Rusli, & Jones, 2020). In addition, there is onion extract (*Allium cepa*) which is also used to help the wound healing process where this onion contains active compounds that play a role in neutralizing and helping the removal of harmful toxic substances (Yunanda, & Rinanda, 2016).

Bananas in addition to being one of the fruit plants with the largest production in Indonesia, also have a variety of types that dominate production in various provinces. East Java Province stands out as the main contributor to banana production, with total production reaching 2.63 tons or 27.36 percent of total banana production in Indonesia, followed by Lampung Province (14.50 percent), West Java (13.72 percent), Central Java (11.30 percent), and South Sumatra (3.48 percent) (Central Bureau of Statistics, 2020).

Not only is the main commodity, but bananas also provide health benefits through various parts of the plant, such as the peel, stem/pseudo stem, and flower (bark). Several previous studies showed that there are bio-active compounds such as flavonoids, lycopene acid, beta carotene, tannins, and saponins contained in bananas that have antibacterial, anti-inflammatory, antineoplastic, hepato-protective, and antioxidant properties (Kraithong, & Issara, 2021; Sowmya, Sree, Patil, & Mehta, 2022; Wang, Jin, Dai, Han, Han, & Bao, 2016; Zulkefli, Che Zahari, Sayuti, Kamarudin, Saad, Hamezah, & Sarian, 2023). In the field of traditional medicine, bananas are also used for the treatment of various diseases, such as diarrhea, diabetes, problems in the intestines, enteritis accompanied by diarrhea and wound healing (Lakshmi, Agarwal, Ansari, Mahdi, & Srivastava, 2014; Wenas, Aliya, & Anjani, 2020).

Wounds as structural or anatomical disorders of a tissue, can occur both as a result of pathological processes from inside and outside the body. This type of wound involves blunt trauma and sharp trauma, each of which results in different types of injuries such as bruises (*contusions)*, abrasions (*abrasions*), tears (*vulnus laceratum*), incisions (*vulnus scissum*), punctures, and stabs (*vulnus caesum*) (Ansori, 2015). This stage involves four phases, namely hemostasis, inflammation, proliferation, and tissue remodeling or resolution. In its phases and physiological functions must occur sequentially, within a certain time, and continue for a certain duration with optimal and appropriate intensity (Dreifke, Jayasuriya, & Jayasuriya, 2015).

Wound healing can be interpreted as the physiological process of the body to normalize the structure and anatomical function of the skin. The wound healing process goes through three stages, namely the inflammatory phase, the proliferation phase, and the maturation phase (Palumpun, Wiraguna, & Pangkahila, 2017). The wound will experience healing failure if there are inhibiting factors. Factors that inhibit wound healing such as infections, hematomas, and foreign bodies. Treatment of wounds aims to reduce risk factors that inhibit wound healing, speed up the healing process, and reduce the wound infections (Amfotis, Suarni, & Arpiwi, 2022)

There are several wound care methods that have been put forward, and the methods used may affect the speed of wound healing. Some examples are wound treatment using 0.9% NaCl (Apriliyasari, & Endro, 2013). Another method is the use of moist dressing (tulle grass product with framycetin sulfate 1%) in a moist state (Erwin, Rusli, & Jones, 2020). Wound care can also be done with certain plants that have specific content and are extracted from plants that become herbal products, one of which is the extract of Kirinyuh leaves. (*Chromolaena odorata*) (Amfotis, Suarni, & Arpiwi, 2022). In addition, there is also Onion extract (*Allium cepa*) (Yunanda, & Rinanda, 2016) and Binahong leaf extract (*Anredera cordifolia (Tenore) Steen*) (Eriadi, Arifin, Rizal, & Barmitoni, 2015).

Based on this background, researchers were interested in finding out the effectiveness of *balbisiana* stem extract in wound care. The aim was to determine the anti-inflammatory activity of *balbisiana* stem extract compared to existing wound care methods, such as *sufratulle*. Thus, this study will provide additional information on more potential and effective wound care methods.

RESEARCH METHOD

This research used explanatory and experimental observation methods using an in vivo control group

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design which was carried out in the laboratory of the Pharmacy Study Program at the Sunan Giri Institute, Ponorogo in November 2023. The samples used were 15 male *Rattus norvegicus* with an average weight of 200 grams (which had been acclimatized in the cage for 7 days by being given A591K ratio type feed). Furthermore, 15 *Rattus norvegicus* were grouped into five treatments, namely 3 mice given sufratulle as a positive control group, 3 mice given gel as a negative control group, 3 mice given 10% *balbisiana* extract, 3 mice given 20% *balbisiana* extract, and 3 mice given 40% *balbisiana* extract.

Preparation of experimental media was carried out by anesthetizing 15 *Rattus norvegicus* using the method (steam) with ether. After that, the back was shaved to a size of 3 cm x 2.5 cm and an incision was made using a sterile scalpel with a depth of 1 mm and a width of 1 mm for a length of 2 cm, then the initial wound was cleaned using 95% alcohol and marked as the incision location. The next stage is to carry out treatment according to the treatment and continue to use the gel dose of 2 ml every 2 days. Observation data is monitored by measuring the length of the wound on days 1, 3, 5, and 7.

The *balbisiana* extract used was extract from 100 grams of dried *balbisiana* banana stems which were soaked in 1 liter of ethanol for 3 days in a closed container, then the solution was filtered through a 50 µm sieve and then dried using a vacuum rotary evaporator at a temperature of 45ºC.

Data were analyzed by measuring the length of the wound, calculating the average value, and determining the percentage of wound healing. Statistical analysis using the Shapiro-Wilk test to test the normality of the distribution of continuous quantitative data and the Levens test to show differences in homogeneity between groups. This research has received permission and recommendations from the Ethical Clearance Committee of the Strada Indonesia Institute of Health Sciences, Kediri, Indonesia with reference number 3946/KEPK/X/2023.

RESEARCH RESULTS

Table 1. The Size of Wound Healing

Treatment	Day 1 (Mean)(SD)	Day 3 (Mean)(SD)	Day 5 (Mean)(SD)	Day 7 (Mean)(SD)	p-value
Balbisiana 10%	15.00±1.000	14.00±1.000	14.00±1.000	12.67±0.577	0.112
Balbisiana 20%	15.33±0.577	13.00±1.000	11.00±1.000	7.00±1.000	0.001
Balbisiana 40%	16.67±0.577	11.33±0.577	7.00±1.000	1.33±1.155	0.000
Sufratulle	15.33±0.577	10.67±1.155	5.00±1.000	1.00±1.000	0.000
Negative Control	16.00±1.000	15.00±1.000	14.67±1.155	13.67±1.155	0.268

Table 1 shows data on wound size, where in the treatment with *balbisiana* 10%, the p-value was 0.112 with mean and standard deviation data of 15.00 ± 1,000 on the first day, 14.00 ± 1,000 on the third day, 14.00 ± 1,000 on the fifth day, and 12.67 ± 0.577 on the seventh day. Furthermore, in the treatment with *balbisiana* 20% a p-value of 0.001 was gained with mean data and standard deviation of 15.33 ± 0.577 on the first day, 13.00 ± 1,000 on the third day, 11.00 ± 1,000 on the fifth day, and 7.00 ± 1,000 on the seven day. Furthermore, in the treatment with *balbisiana* 40% a p-value of 0.000 was obtained with mean data and standard deviation of 1.67 ± 0.577 on the first day, 11.33 ± 0.577 on the third day, 7.00 ± 1,000 on the fifth day, and 1.33 ± 1,155 on the seventh day. Meanwhile, in the treatment with *sulfratulle* (positive control) the p-value was 0.000 with mean data and standard deviation of 15.33 ± 0.577 on the first day, 10.67 ± 1.155 on the third day, 5.00 ± 1.000 on the fifth day, and 1.00 ± 1,000 on the seventh day. Meanwhile, the negative control obtained a p-value of 0.268 with mean and standard deviation data of 16.00 ± 1,000 on the first day, 15.00 ± 1,000 on the third day, 14.67 ± 1.155 on the fifth day, and 13.67 ± 1.155 on the seventh day.

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Tabel 2 Games Howell Post-hoc Test

Treatments	p-value				
	Balbisiana 10%	*Balbisiana* 20%	*Balbisiana* 40%	Sufratulle	Negative Control
Balbisiana 10%	---	0.197	0.101	0.030	0.375
Balbisiana 20%		---	0.708	0.366	0.043
Balbisiana 40%			---	0.991	0.042
Sufratulle				---	0.012
Negative Control					---

Based on the post-hoc test results in table 2, it shows that the difference between the p-value of *balbisiana* 10% and *balbisiana* 20% is 0.197, with *balbisiana* 40% is 0.101, with *sulfratulle* is 0.030, and with the negative control is 0.375. Meanwhile, the difference between the p-value of *balbisiana* 20% and *balbisiana* 40% is 0.708, with *sulfratulle* 0.366, and with the negative control 0.043. Meanwhile, the difference between the p-value of *balbisiana* 40% and *sulfratulle* is 0.991, and with the *negative control* 0.042. Furthermore, the difference between the p-value of *sulfratulle* and the *negative control* is 0.012.

DISCUSSION

In this study, experimental actions were carried out on days 1, 3, 5, and 7, and the healing observation process started from the following day of the treatment, namely days 3, 5, and 7. Based on the research results, it was seen that the administration of *balbisiana* extract accelerated the wound healing process in *Rattus norvegicus*. With *balbisiana* extract treatment, wound healing is quite good. The results showed that mice that received *balbisiana* extract at a concentration of 40% showed a more optimal rate of wound healing compared to the other three groups, almost resembling the results of the positive control treatment with *sufratulle*. This was proven by observing incision wounds in the 40% *balbisiana* group and the positive control with *sufratulle* in a p-value of 0.000, where it showed that it had a significant effect on wound healing. Apart from that, the observation data showed that there was a wound healing process with the skin becoming dry, the color of the wound resembling normal skin, there were no signs of infection (pus/exudate). There was no necrotic tissue, no erythema, no edema, and changes in the length of the wound which indicates that the wound healing process is ongoing.

The active substances contained in *balbisiana* stem extract include flavonoids, alkaloids, tannins, saponins and polyphenols. Compounds from the flavonoid and phenol groups have a role as antioxidants, both through the provision of hydrogen atoms and through their ability to form complexes with metals. These compounds can be in the form of *glucosides* (containing glucose chains) or in free form called *aglycones* (Nurhaeni, 2019). Alkaloid compounds, which have base-like properties and at least one nitrogen atom in their heterocycles, can reduce inflammation and colon damage in various models of colitis (Peng, Zheng, Li, Liang, Wang, Huang, & Xi 2019). Saponin acts as an anti-fungal agent by reducing the surface tension of the sterol membrane in the *Candida albicans* cell wall thereby increasing its permeability. Increased permeability causes thicker intracellular fluid to escape from the cells, resulting in the death of *Candida albicans*. Another component is tannin, a complex compound in the form of natural polyphenols found in plants. Tannins are divided into two groups, namely hydrolyzed tannins (*ellagitannins* and *gallotannins*) and condensed tannins (*proanthocyanidins*) (Sepnoor, Carabelly, & Apriasari, 2013). Tannins play a role in the blood clotting process which results in the buildup of fibroblast tissue and prevents infections that can be caused by bacteria around the wound area. Meanwhile, saponins can reduce cell surface pressure and cause cell lysis (Kayalvizhi, & Usha, 2014).

CONCLUSION

Based on the results of this study, it can be concluded that *balbisiana* extract 10%, 20%, and 40% can help the wound healing process in *Rattus norvegicus*. The highest concentration in this study was *balbisiana* extract 40% showed activity

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resembling *sufratulle* activity in helping the wound healing process which was better than other concentrations.

SUGGESTION

There are still some limitations in this study. Hence, it is suggested for further research to deal with a larger number of samples to generate more extensive data, allowing the use of alpha 0.01.

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