The Effect of *Rhizophora apiculata* Bark Ethanol Extract on Burns Healing of *Rattus norvegicus* Sprague Dawley Strain

Syazili Mustofa[1][a], Delisa Mutiara Nabila[2] and Evi Kurniawaty[1]
[1]*Department of Biochemistry and Molecular Biology, Medical Faculty, Universitas Lampung, Prof. Soemantri Brojonegoro Street, Bandar Lampung, Indonesia*
[2]*Medical Faculty, Universitas Lampung, Bandar Lampung, Indonesia*

Keywords: Burn Wound, *Rhizophora apiculata* Bark Extract, Wound Healing.

Abstract: This study aimed to evaluate the ability of an ethanolic extract of *Rhizophora apiculata* bark to treat burn wounds in rats. Second-degree burn wounds were induced in five groups of six rats each, and topical treatment was done daily for 26 days. Group KN and K+ received aqua bidestilata and bioplacenton® (containing placenta extract 10% and neomycin sulfate 5%) as a control and reference standard, and group P1, P2 and P3 were given ethanolic *Rhizophora apiculata* bark extract of 20%, 30% and 40% respectively. The observing burn wounds for ± 26 days were enrolled for evaluating the effects on the burn healing phase, burn area reduction, and burn healing time. The area of burn wounds in the groups of rats given the extract began to shrink more quickly on the 15th day of observation. The One-way ANOVA test revealed p=0.001, and the LSD Post Hoc test revealed $p < 0.05$, indicating a significant difference in the area of burns on day 15 for all treatment groups. The healed wounds in extracts-treated rats were faster and had better wound healing time. Wound contractions were relatively better in extract groups. In conclusion, the *Rhizhopra apiculata* bark ethanolic extract positively affected wound burn healing activity.

1 INTRODUCTION

Burns ranks fourth among the world's most common injury categories. The World Health Organization (WHO) estimates that 180,000 people die as a result of burn injuries out of an estimated 11 million people who suffer from burn wounds annually. Burns are complex wounds with a high mortality rate that are challenging to heal, regardless of the underlying cause (Opriessnig, 2023).

Burn injuries are generally categorized as a type of wound where the source is one of several factors, including heat, cold, electricity, chemicals, friction, or radiation. Conversely, wound healing is a complicated process, and knowledge of its biological trend and the variations in how various wounds heal can often lower the danger and significantly lessen the likelihood of further harm to the injured tissue and other organs. Burn wound healing is a dynamic process involving various interactions of cytokines and extracellular matrix. This healing process is divided into three phases: inflammation, proliferation, and tissue remodelling. Wound healing consists of wound healing and contracture, where regeneration of the epithelial layer occurs and the wound shrinks (Żwierełło, 2023). Healing burn wounds is still a challenge faced by the modern medical era. Only a few drugs can speed up wound healing. There is still a need to find new drugs to speed up the healing of burn wounds. Medicines derived from medicinal plants have great potential to be developed as alternative treatments (Huang, 2022).

Rhizophora apiculata, commonly known as Bakau Minyak in Indonesia, is naturally distributed in Southeast Asia and India. The plant contains saponin, tannins, flavonoids and terpenoids (Bulan, 2022). Traditionally, *Rhizophora apiculata* is employed by Indonesians as a medicinal plant for gastrointestinal and skin diseases. Our earlier research revealed that this plant might have antioxidant (Mustofa, 2023) and anti-inflammatory properties (Mustofa, 2019). For these reasons, we were interested in researching the effect of *Rhizophora apiculata* on healing burns.

[a] https://orcid.org/0000-0002-7646-0869

Mustofa, S., Nabila, D. M. and Kurniawaty, E.
The Effect of Rhizophora apiculata Bark Ethanol Extract on Burns Healing of Rattus norvegicus Sprague Dawley Strain.
DOI: 10.5220/0013668300003873

In *Proceedings of the 1st International Conference on Medical Science and Health (ICOMESH 2023)*, pages 223-227
ISBN: 978-989-758-740-5

2 MATERIALS AND METHODS

2.1 Plant Material

The bark was obtained from the Forest Management Unit KPH Gunung Balak, East Lampung, Indonesia, in June 2022. The plant was authenticated by the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung.

2.2 Preparation of Extracts

We have carried out the process of making ethanol extract in two steps, namely maceration and evaporation. Maceration is the process of soaking simplicia using a solvent for several days at room temperature and protection from light. After air drying, the bark was ground into a fine powder with a blender. We weighed 100 grams of *Rhizophora apiculata* stem bark powder and put it in a 2-litre Erlenmeyer glass. Next, soaking was done with 2000 ml of 95% ethanol solvent and stirred for approximately 30 minutes until the powder was dissolved entirely. Finally, this solution was left to settle for 18 hours. After this process, the second stage is carried out, namely evaporation. This process uses a rotatory evaporator and water bath (temperature 90˚C). The ethanol solution and active substance in the flask are allowed to separate until the ethanol flow stops dripping into the collection flask (Mustofa, 2020).

2.3 Animals Model

For this experiment, male rats weighing 200–250 g were employed. They were housed in a central animal house with a temperature of 23 ± 1o C and a 12-hour light/dark cycle. They were fed adequately and appropriately and had unlimited access to water (Kurniawaty, 2022). Before beginning this experiment, the medical faculty of Universitas Lampung approved the ethical clearance for the research by number 4479/UN26.18/PP.05.02.00/2022.

2.4 Burn Induced

Thirty rats underwent a back shave, 70% ethanol disinfection, and xylazine and ketamine injections to induce anaesthesia. Afterwards, an iron plate with a 20 mm diameter that had been heated over an electrical heater for 10 seconds was used to create a circular burn wound (3.14 cm2) on their dorsal portions. After that, distilled water was applied to the wound for a minute.

2.5 Experiment Protocol

Burn induced Rats were split into five groups (n =6): group KN got aqua bidestilata as control, group K+ received reference standard treatment (Bioplacenton®), groups P1, P2, and P3 received 20%, 30%, and 40% (w/w) of the extract, respectively. Rats' dorsal back wounds were treated topically with aqua bidestilata, Bioplacenton®, and the extract every day at 24-hour intervals. The day of burn generation was zero, and a 26-day treatment protocol was initiated 24 hours after the burns were created. Wound care was given once a day, and the burn wound was cleaned first with 0.9% NaCl, then dried and covered using sterile gauze.

Figure 1: Induced wound burns on experiment Rats.

2.6 Macroscopic Wound Analysis

Burn wounds are treated until they heal, characterized by scarring or scabbing and tightening and closing the wound. Wound healing was assessed by observing for 26 consecutive days using 0.01 mm scale callipers (see Figure 1). The area of a burn wound was calculated by measuring the average wound diameter from four positions and then putting it into the ¼.π.d² formula for circle size.

Analytical statistics Mean ± S.D. was used to express all values. After one-way ANOVA data analysis, a post hoc test was performed. At $p<0.05$, the results were deemed to be statistically different.

3 RESULTS

We have conducted a qualitative test of the extract in the Medical Biochemistry laboratory of the Faculty of Medicine, Universitas Lampung. This extract

contains various active substances, as shown in Table 1.

Table 1: Qualitative phytochemical screening of ethanolic extracts of *Rhizophora apiculata* bark

Qualitative active compound	Result
Saponin	Positive
Steroid	Negative
Terpenoids	Positive
Tannins	Positive
Alkaloids	Negative
Flavonoid	Positive

The size of burn wounds on day 4 in all groups of rats was 3.14 cm2, still the same as the initial day of treatment. There was no extensive reduction, and inflammation in the form of redness was obtained. The area of the burn wound on the 10th day appeared to be reduced by 15-20% of the original size. However, the mean area of burn wounds for each group of rats was not significantly different. On day 15, treatment groups P2 and P3 had quite different macroscopic healing compared to the control group (p-value = 0.001). It shows that administering *Rhizophora apiculata* extract has a good effect on wound healing (see Table 2).

Table 2. Results of measuring the average area of burn wounds per day.

Experiment day	Average burn area (cm^2)				
	KN	K+	P1	P2	P3
4^{th}	3.14	3.14	3.14	3.14	3.14
10^{th}	2.7	2.8	2.9	2.7	2.4
15^{th}	1.4	1.3	1.4	0.8*	0.7*
21^{th}	0.17	0.12	0.64	0*	0*

(* significantly difference compared to control group)

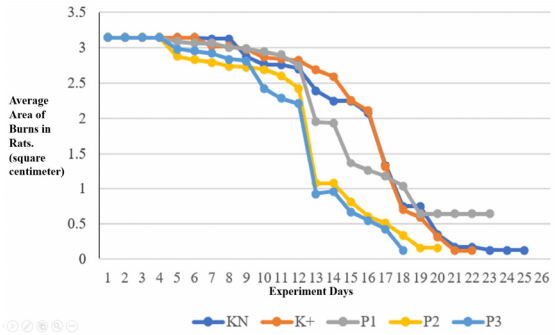

Figure 2: the average area of burns on each day of the experiment.

The reduction in the area of burn wounds occurred more quickly in the group of mice given the extract. Burns were no longer visible on day 18 in group P2 and day 19 in group P3. Meanwhile, burns were no longer visible on day 21 in the control group (see Figure 2).

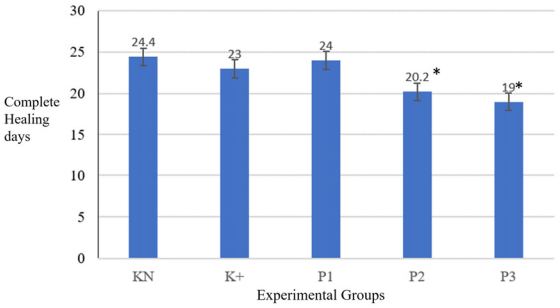

Figure 3: The averages complete burn wound healing time.

The results of macroscopic observations found that the groups given the extract, namely P2 and P3, experienced faster wound healing, namely on day 19 and day 20. Meanwhile, the control group only experienced the healing of burn wounds on days 23 and 24 (see Figure 3).

4 DISCUSSIONS

The complicated process of burn healing causes dermal and epidermal tissue degradation. It triggers a few physiological reactions at the injury site, such as an immediate inflammatory response followed by a protracted, intense tissue creation phase. Granulocytes or Polymorphonuclear leukocytes leucocytes (PMNs) mediate this acute inflammation. In this stage, the inflammatory site produces free radicals that have the potential to harm tissues and hinder the healing process of wounds (Żwierełło, 2023).

Mast cells and macrophages are immune cells involved in the inflammatory phase. Numerous growth factors and cytokines are secreted by these two cells. Nerve Growth Factor (NGF) is a significant cytokine that contributes to inflammatory responses. Next, during the proliferation phase, NGF causes endothelial cells to express Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF), increasing keratinocyte proliferation and angiogenesis (El Baassiri, 2023).

Therefore, tissue development, re-epithelization, and epidermis differentiation can be hastened using an anti-oxidant to minimize inflammation. Preventing some of the frequently fatal infections in the wound area is another critical step in healing. The antibacterial action can promote wound healing and

partially inhibit the growth of pathogenic microbes on the skin. Thus, natural ingredients of antibacterial, anti-inflammatory, and antioxidant properties can be fundamental in the healing process of burns (Huang, 2023). The extract of *Rhizophora apiculata* prevented rat hepatocyte and pancreatic cell necrosis caused by cigarette smoke in an animal model assessment of its anti-inflammatory action in rats (Mustofa, 2018). Moreover, *Rhizophora apiculata* possesses a broad antibacterial activity (Acharya, 2023). Thus, the antibacterial, anti-inflammatory, and anti-inflammatory properties of *Rhizophora apiculata* may partially explain its favourable benefits in burn healing acceleration and inflammation reduction (Nisar, 2019). These effects could be attributed to phytochemical components. Previous research and our phytochemical analysis indicate that terpenoids, flavonoids, tannins, and saponins are some of the significant constituents of *Rhizophora apiculata* (Chan, 2022).

One way to think of phenolic phytochemicals as the main classes of secondary metabolites with antimicrobial properties is flavonoids and tannins. Through various mechanisms, including their astringent, antibacterial, antioxidant, and angiogenic effects, tannins can hasten the healing of wounds (Ramya 2023). Terpenoids promote the breakdown of stored extracellular proteins and prevent prostaglandin formation, which can reduce tissue oedema and inflammation. Because of their antioxidant potential, they can avoid damage caused by free radicals, just as flavonoids do through other processes such as direct radical scavenging (Chan, 2022).

Because they regulate inflammation-related cells and have an antioxidative action, flavonoids have anti-inflammatory properties in vivo. Additionally, reducing lipid peroxidation can improve collagen strength and viability, boost vascularity and circulation, and stop cell damage and necrosis. These arguments suggest that aqueous extract's superior antioxidant and anti-inflammatory properties may contribute to its comparatively more significant effect on burn healing, but they are not the only ones. The results of the qualitative phytochemical screening indicated that the ethanolic extract contained more flavonoids. Thus, the higher flavonoid content of the ethanolic section explains its more decisive antioxidant action (Nisar, 2019). However, further quantitative experiments are needed for a more accurate assessment.

5 CONCLUSIONS

Rhizophora apiculata extracts significantly accelerated the healing of burn wounds in rats. This effect may result from several mechanisms, including a faster rate of vascularization and re-epithelialization, the inhibition of harmful free radicals, a decrease in oedema and inflammation, and the ability of this plant's antioxidant, anti-inflammatory, and antimicrobial components to control infection. More research utilizing purified components is necessary to fully comprehend the mechanism underlying the burn-healing ability of *Rhizophora apiculata*.

ACKNOWLEDGEMENTS

This research was financially supported by HETI Project Universitas Lampung.

REFERENCES

Acharya, S., Jali, P., Pradhan, M., Pradhan, C., & Mohapatra, P. K., 2023. Antimicrobial and Antioxidant Property of a True Mangrove Rhizophora apiculata Bl. *Chemistry & Biodiversity*, *20*(9), e202201144.

Bulan, D. E., Nurfadilah, N., Syahrir, M. R., Mismawati, A., Torambung, A. K., & Rachmawati, M., 2022. Phytochemical Composition and Antioxidant Activity of Leaf Extracts from Three Rhizophora Species from Bontang Waters, Indonesia. *Tropical Journal of Natural Product Research*, *6*(8).

Chan, E. W. C., Lim, W. Y., Wong, C. W., Ng, Y. K., 2022. Some notable bioactivities of Rhizophora apiculata and Sonneratia alba. *ISME/GLOMIS Electron J*, *20*(4), 23-6.

El Baassiri, M. G., Dosh, L., Haidar, H., Gerges, A., Baassiri, S., Leone, A., Jurjus, A., 2023. Nerve growth factor and burn wound healing: Update of molecular interactions with skin cells. *Burns*, *49*(5), 989-1002.

Huang, W., Wang, Y., Tian, W., Cui, X., Tu, P., Li, J., ... & Liu, X., 2022. Biosynthesis investigations of terpenoid, alkaloid, and flavonoid antimicrobial agents derived from medicinal plants. *Antibiotics*, *11*(10), 1380.

Kurniawaty, E., Megaputri, S., Mustofa, S., Rahmanisa, S., Audah, K. A., & Andriani, S., 2022. Ethanol extract of Bruguiera gymnorrhiza mangrove leaves and propolis activity on macroscopic healing of cuts in vivo. *Acta Biochimica Indonesiana*, *5*(1), 94-94.

Nisar, A., 2019. Identification of Flavonoids from the Leaves Extract of Mangrove (Rhizophora apiculata). *Recent Adv Biol Med*, *5*(2019), 9451.

Mustofa, S., Bahagia, W., Kurniawaty, E., Rahmanisa, S., & Audah, K. A., 2018. The effect of mangrove

(Rhizophora apiculata) bark extract ethanol on histopathology pancreas of male white rats Sprague Dawley strain exposed to cigarette smoke. *Acta Biochimica Indonesiana*, *1*(1), 7-13.

Mustofa, S., & Hanif, F., 2019. The protective effect of Rhizophora apiculata bark extract against testicular damage induced by cigarette smoke in male rats. *Acta biochimica indonesiana*, *2*(1), 23-31.

Mustofa, S., Ciptaningrum, I., Zuya, C. S., 2020. Subacute toxicity test of Rhizophora apiculata bark extract on liver and pancreas histopathology of rats. *Acta Biochimica Indonesiana*, *3*(2), 89-97.

Mustofa, S., Tarigan, C. Y., 2023. Efek Protektif Ekstrak Kulit Batang Bakau Rhizophora apiculata terhadap Kerusakan Histologi Paru Rattus norvegicus yang Diinduksi Asap Rokok. *Jurnal Kesehatan*, *14*(2), 241-250.

Opriessnig, E., Luze, H., Smolle, C., Draschl, A., Zrim, R., Giretzlehner, M., ... & Nischwitz, S. P., 2023. Epidemiology of burn injury and the ideal dressing in global burn care–Regional differences explored. *Burns*, *49*(1), 1-14.

Ramya, R., Kamoona, S., Hatta, F. A. M., Sulaiman, W. S. H. W., Latiff, N. H. M., Othman, R., 2023. A Study on an Active Functional Group and Antimicrobial Properties From Rhizophora apiculata Extracts Used in Traditional Malay as Medicine. *Malaysian Applied Biology*, *52*(4), 153-160.

Żwierełło, W., Piorun, K., Skórka-Majewicz, M., Maruszewska, A., Antoniewski, J., & Gutowska, I., 2023. Burns: classification, pathophysiology, and treatment: a review. *International journal of molecular sciences*, *24*(4), 3749.