Burns Wound-Healing Activity in Male White Rats of Matoa Stem Bark(Pometia pinnata) Ethanolic Extract

by Iik Bhakti Wiyata Kediri Perpustakaan 1

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Burns Wound-Healing Activity in Male White Rats of Matoa Stem Bark (*Pometia pinnata*) Ethanolic Extract

Fresi Lilianturi, Shofiatul Fajriyah*, Fenita Shoviantari

Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Kediri, East Java, Indonesia

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*Corresponding author e-mail: shofiatul.fajriyah@gmail.com

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ABSTRACT

***Introduction**: Burns are a global public health problem. The activity of herbal products in stimulating burn wound healing is supported by many animal studies and few clinical trials. The people of Papua New Guinea use the chewed bark of the matoa tree to heal burns. Matoa's stem bark contains flavonoids, terpenoids and tannins that promote wound healing. These compounds have antimicrobial, antioxidant and wound healing properties. They increase blood clotting, fight infection and accelerate wound healing. **Aims**: The aim of this research was to determine whether ethanol extract of Matoa stem bark has anti-burn activity against male white rats of the Sprague Dawley strain. **Method:** In this study, a method was used to measure the area of burn wound in 5 treatment groups for 14 days. Group I were treated with the MEBO ointment (the positive control group); Group II with vaseline flavum (the negative control); Group III was given Matoa bark extract ointment at a concentration of 10%; group IV at a concentration of 20%, while group V at a concentration of 30%. **Result:** Qualitative analysis of chemical compounds shows that the ethanolic extract of Matoa stem bark contains flavonoids, triterpenoid tannins, and saponins. Ethanol extract of matoa bark 20% and 30% had burn wound healing activity in male Sprague-Dawley white rats. Activity was significantly different from negative control and not significantly different from positive control. **Conclusion:** A 20% and 30% ethanolic extract of the stem bark Matoa is considered a topical agent for use in treating burns wound.*

***KEYWORDS**: Matoa, stem bark, burn, wound healing, rat*

INTRODUCTION

Burns are a global public health problem. They cause about 180 000 deaths each year. The majority of these occur in low- and middle-income countries. Nearly two-thirds occur in the WHO region of Africa and South-East Asia. Non-fatal burns are a major cause of morbidity, including prolonged hospitalisation, disfigurement and disability, and often lead to stigma and denial. (WHO, 2018). At the Ciptomangunkusumo Hospital in Jakarta, there were 709 burn patients over a five-year period, with an average of 141 patients per year and an increase of 171 per cent from 2013 to 2017 (Wardhana & Winarno, 2020). First-degree burns (superficial, affecting only the epidermis) are usually harmless, very painful, heal without scarring and do not require surgery. The skin is red and the duration of pain is limited. Burns that extend to the underlying layer of skin (dermis) are called partial or second-degree

burns. These burns often cause painful blisters, require dressing and wound care, and may scar, but do not require surgery. (Jeschke et al., 2020).

Topical treatment plays an important role in healing burn wounds. The SSD (Silver Sulphadiazine) is a standard topical antibiotic used to prevent secondary infections in burns. Secondary infections cause delayed healing, increased scarring complications, and even invasive infections leading to death. There is no evidence to support the use of topical antibiotics for the prevention of secondary infections (Dien et al., 2015). There is currently no ideal topical antimicrobial that can be recommended for treating burns (Garcia et al., 2022). Only in situations such as superficial wounds or wounds on the face are recommendations made for the use of topical antibiotics. The use of topical antibiotics in burn patients is even more likely to lead to antimicrobial resistance, based on systematic reviews (Norman et al., 2017). There is a relationship between overuse and misuse of antibiotics with higher resistance to pathogenic organisms, according to a meta-analytic study conducted in Iran that compared the antibiotic resistance profiles of burn patients in Iran (Javanmardi et al., 2019). Neglecting the research for new natural substances and relying on synthetic compounds may have led to the loss of many potentially useful drugs, especially those related to new anti-infectives against the increasing number of antibiotic-resistant microbes (Sevgi et al., 2014). There is therefore a need for further research into other alternative treatments for burns. Topical herbal preparations can be used to treat minor burns, such as MEBO®. There is evidence that MEBO® is as effective as SSD treatment (Dien et al., 2015).

Herbal products have been used in the treatment of burns for centuries. The activity of herbal products in stimulating burn wound healing is supported by many animal studies and a few clinical trials. The topical application of herbal products with anti-microbial, anti-inflammatory and anti-oxidant activity seems to be a good alternative for the treatment of burn wounds. (Herman & Herman, 2020). The people of Papua New Guinea use the chewed bark of the matoa tree to heal burns (Thomson & Thaman, 2006). The stem bark of Matoa contains chemical compounds such as flavonoids, saponins, tannins, triterpenoids, steroids and phenolic compounds, which have strong antioxidant activity (Rahmawati et al., 2021). Phytochemicals have great potential for treating microbial infections and wounds. Compounds that have antimicrobial, antioxidant and wound healing activities promote blood clotting, fight infection and accelerate wound healing (Selvakumar et al., 2018). In a rat model, tannins can accelerate wound healing, re-epithelialization and hair follicular growth by upregulating the expression of growth factors such as basic fibroblast growth factor (bFGF), transforming

growth factor beta (TGF-beta) and VEGF; downregulating inflammatory cytokines such as IL-1 and IL-6; and activating the ERK 1/2 pathway (Jing et al., 2022). Flavonoids are thought to accelerate wound healing by decreasing oxidative stress in wounds (Zulkefli et al., 2023)

Herbal preparations can compete with conventional medicine in the treatment of burns, with different mechanisms of action, antibacterial activity and safety. The increasing interest in alternative therapies and herbal medicine has also increased the demand for herbal products. The development of topical preparations for burns that can replace the general use of antibacterial agents is a challenge. Many studies show that herbal-based formulations provide strong competition to synthetic compounds (Skowrońska & Bazylko, 2023). In view of the above, the effect of application of Matoa bark (*Pometia pinnata*) ethanol extract ointment on the healing of burns in male Sprague Dawley white rats (*Rattus novergicus*) was studied scleroderma, diabetes and an unhealthy lifestyle (Feller & Lemmer, 2012; Irani, 2020; Miloro et al., 2011; Petti, 2009; Scully & Bagan, 2009).

MATERIAL AND METHODS

This research is an experimental study that examined the activity of 70% ethanol extract of matoa bark on the healing of superficial burns wound in male white Sprague Dawley rats. In this study, a method was used to measure the area of the wound in 5 treatment groups for 14 days.

The materials used in this research were matoa bark obtained from Blitar Regency, 70% ethanol, glacial acetic acid, concentrated sulphuric acid, Mg powder, methanol concentrated HCl, 1% $FeCl_3$, Liebermann-Burchard reagent, distilled water, MEBO® ointment mild burn treatment produced by Combhipar, petroleum jelly, lidocaine. The experimental animals used were male white rats (*Rattus novergicus*) of the Sprague-Dawley strain, aged 2-3 months and weighing 200-300 g, obtained from the UGM Yogyakarta Experimental Animal Development Unit.

Preparation of Matoa Stem Bark Extract (MBEE)

The identification of matoa bark plants was carried out at the Bhakti Wiyata Kediri Institute of Health Sciences. One kilogram of matoa bark, stripped of any adhering dirt, then washed with running water. It was then dried in the open air and protected from direct sunlight until it was dry. The dried samples are crushed in a blender and sieved. The fine powder is stored in a sealed container away from sunlight. It was processed into simplicia powder. By maceration with 4 L of 70% ethanol for three days, 400 grams of simplicia powder was extracted. This was followed by the addition of 1 L of ethanol to the residue of the first maceration and remacerated it for three days. The liquid extract obtained was

then concentrated in a water bath at a temperature of 40 °C. A thick extract was obtained, and the yield was 49.80% (Aryantini et al., 2023) .

Chemical screening

A chemical screening was carried out on the ethanolic extract of the stem obtained matoa. Flavonoid test on the extract using Mg and HCl powdered reagents. Tannin tested the extract using 1% $FeCl_3$ solution reagent. The triterpenoid test is carried out by dissolving the sample with Liebermann Burchard's reagent. The saponin test is indicated by the formation of a stable foam (Hanani, 2016).

Preparation of matoa bark extract ointment

The ethanolic extract of the stem bark of Matoa was prepared into an ointment with a concentration of 10 %, 20 % and 30 %. Matoa stem bark ethanol extract was mixed with Vaseline flavum, total weight 0.5 g. 0.5 grams was used for 5 mice (25 burn areas) and was used only once a day. The next day a new ointment was made with the same weight. The same treatment was carried out until the 14th day (Dwita et al., 2019).

Creating burn wounds in rats

The site of the burn was on the skin on the back of a male Sprague Dawley white rat. The hair is shaved with a razor and a 3 x 3 cm square area is made. Firstly, disinfect skin with 70% alcohol. An intramuscular injection of 0.2 ml lidocaine is then administered to induce anaesthesia. Three minutes later burns were produced by applying a 10 mm diameter stainless steel plate which was heated in boiling water (temperature 100°C) for 5 seconds (Cai et al., 2014).

Burns wound healing activity test

There were 5 rat in use. Each rat had 5 burns on its back and received 5 treatments. This means that there were 5 groups to treat. Group I was a group of rat that were treated with the MEBO ointment (the positive control group). Group II consisted of rat which were Vaseline flavum (negative control). Group III was given Matoa bark extract ointment at a concentration of 10%, group IV at a concentration of 20%, while group V had a concentration of 30%. The experiment was carried out for 14 days. The ointment was applied to the test group once a day. 0.1 gram was used for one application. The progress of wound healing was observed macroscopically, the wound diameter was measured and the surface area of the burn wound was calculated for 14 consecutive days. Wound diameter was measured in different directions. The Morton method was used (Sumoza & Rahayu, 2014).

$$dx = \frac{dx(1) + dx(2) + dx(3) + dx(4)}{4}$$

Where dx is wound diameter on day x (mm), dx (1), (2), (3), (4) is wound diameter was measured from various directions.

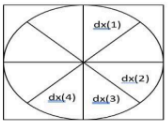

Figure 1. Diameter measurement of a wound

Table 1. Screening Test Results for Matoa Bark Extract

Test	Result
Flavonoid	+
Tanin	+
Triterpenoid	+
Saponin	+

For example, measure the diameter of the burn wound in one of the burn wound areas treated with the positive control on the first rat on day 14. If the diameter is measured from 4 different directions and found that diameter 1 is 4.18 mm, diameter 2 is 4.19 mm, diameter 3 is 4.19 mm and diameter 4 is 4.19mm, then the average (dx) is 4.19.

$$dx = \frac{4{,}18 + 4{,}19 + 4{,}19 + 4{,}19}{4} = 4{,}19$$

Calculate the area of the burn wound using the circular formula L= $\frac{1}{4}\ x\ 3{,}14\ x\ dx^2$

Then calculated the average area of burn wounds on the five rat with the same treatment Calculation of the percentage of burn wound healing is carried out using the following formula:

Percentage of burn wound healing = $\frac{L1-L14}{L1} x\ 100\%$

L1: Average area of the burn wounds in 5 rat on the first day of treatment

L14: Average area of the burn wounds in 5 rat on the 14th day of treatment.

Data analysis.

The research results obtained were a decrease in burn wound diameter and surface area. These results were analysed using the One Way Anova statistical test. The one-way ANOVA test is carried out in 4 steps, including: the normality test, the homogeneity test, the ANOVA test and the post hoc test.

RESULTS AND DISCUSSION

Research on burn wound healing tests of ethanolic extract of Matoa bark on male white rats (*Rattus norvegicus*) of Sprague Dawley strain has been declared ethically acceptable by the Research Ethics Committee of the Bhakti Wiyata Kediri Institute of Health Sciences under number 92/PP2M-KE/I/2020. The results of the determination of the Matoa plant showed that matoa belongs to the species Pometia pinnata J.R & G.Forst. The screening results of the ethanol extract of matoa stem bark showed that the extract contained flavonoids, tannins, triterpenoids and saponins (Table 1).

The qualitative analysis of the chemical compounds indicates that the ethanolic extract obtained from the stem bark of Matoa contains flavonoid compounds, triterpenoid tannins and saponins. The results of this research are consistent with previous studies indicating that pentacyclic triterpenoids, in particular, are present in the ethanolic extract of Matoa stem bark. (trimedona, 2015). Other studies have concluded that ethanol extract from matoa bark contains saponins, triterpenoids, tannins and flavonoids (Mataputun et al., 2013; Rahmawati et al., 2021). The bark of the Matoa tree contains polyphenols, which are antioxidant compounds (Prihanti et al., 2020).

Table 2. Percentage of burn wound healing

Treatment	Diameter of wound in days (mm) 1	7	14	% wound healing	Surface reduction
MEBO ointment	85.48	44.18	17.72	79.27	271.04*
Negativ control	83.96	75.99	51.19	39.03	131.08
MBEE 10%	92.39	65.40	36.94	60.02	221.80*
MBEE 20%	93.61	58.48	24.10	74.26	287.04*a
MBEE 30%	93.72	45.04	15.71	82.87	304.03*a

n = 5; * means significant different from negative control ($p<0.05$), [a] means no siginicant different ($p>0.05$)

The percentage of healing can be determined by the reduction in the size of the burnt area in days 1 to 14 (Fig. 2). The positive control showed a result of 79.27% while the negative control showed 39.03%. The 10% extract showed a percentage of 60.02%, the 20% extract 74.86% and the 30% extract 82.87% (Table 2).

The research data were found to be normally distributed and homogeneous. One way ANOVA was then used to test whether there were differences between the treatment groups and LSD was perform a post hoc test. The results of the one way ANOVA test for the treatment group on the reduction of the burn area were found to have a significance value of 0.003. A significance value <0.05 indicates a significant difference. Table 2 shows that matoa stem bark ethanol extract treatment group with concentrations of 20% and 30% differed significantly from negative control. There was no significant difference between the treatment groups of ethanol extract of Matoa stem bark with concentrations of 20% and 30% compared to the positive control. MEBO ointment was used as the positive control. MEBO accelerates wound healing and increases neovascularisation in skin excision wounds through the promotion of granulation tissue development. MEBO improves skin excision wound healing and is recommended for slow healing skin wounds. (Abdullah & Rejab, 2022).

Matoa stem bark ethanol extract concentrations of 20% and 30% have significant burn wound healing activity compared to the negative control. Topical application of herbal products to heal burns exhibits antimicrobial, anti-inflammatory and antioxidant activities. (Herman & Herman, 2020). Research by Prihanti et al 2020 shows that Matoa stem bark extract has antioxidant activity, as indicated by reduced serum MDA in Wistar rat. (Prihanti et al., 2020). Matoa stem bark methanol extract has strong antioxidant activity with IC50 = 70.93 ppm when tested using DPPH (Nabilah & Sutoyo, 2019).

Matoa bark extract has an analgesic effect in mice that is induced by heat stimulation. (Lumintang et al., 2015). The results of the screening of the ethanolic extract of the stem bark of matoas revealed the presence of flavonoids, tannic acids, triterpenoids and saponins. Examples of compounds involved in the healing process of burn wounds are flavo-

Figure 2. The reduction size of the burnt area

noids, tannins, triterpenoids and saponins. (Skowrońska & Bazylko, 2023). According to research by Tambingon et al 2023, a topical cream preparation of matoa stem bark extract has antibacterial activity against the bacterium Staphylococcus aureus, with a moderate level

of inhibition. (Tambingon et al., 2023). Staphylococcus aureus is one of the most common bacteria causing infection in burn patients. (Chaudhary et al., 2019; Zhou et al., 2023). Therefore, an ethanolic extract of the stem bark of the matoa at a concentration of 30% can be considered as a topical preparation to aid in the healing of burns wound.

CONCLUSION

Matoa stem bark ethanol extract 20% and 30% had burn wound healing activity in male white Sprague-Dawley rats. The activity was significantly different from the negative control group and not significantly different from the positive control group. An ethanolic extract of the stem bark of the matoa at a concentration of 30% can be considered as a topical preparation to aid in the healing of burns wound.

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