

The effectiveness of tamarind leaf extract (*Tamarindus indica* L) in wound healing in female white rats (*rattus norvegicus*)

Nurfaidah^{1*}, Elly wahyudin², Mardiana Ahmad³, Prihantono⁴, Stang⁵

¹Department of Midwifery Post Graduate School, Hasanuddin University, Makassar, Indonesia

²Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia

³Department of Midwifery Post Graduate School, Hasanuddin University, Makassar, Indonesia

⁴Department of Public Health, Faculty of Public Health, Makassar, Indonesia

⁵Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

Abstract

Tamarindus indica L. leaves are rich in bioactive compounds and have traditionally been used for wound treatment, but scientific validation is still limited. This study aims to test the effectiveness of ethanol extract of tamarind leaves (*Tamarindus indica* L.) on the wound healing process in female white rats (*Rattus norvegicus*). A true experimental design with a post-test control group was conducted on 30 female *Rattus norvegicus* and divided into six groups (n = 5). Treatments included a base ointment, Betadine extract ointment, Bioplasenton, and *Tamarindus indica* at concentrations of 10%, 15%, and 20%. Wound healing was assessed daily using the REEDA scale and confirmed histologically on day 14. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. The study showed that the 20% tamarind leaf extract ointment group had the fastest healing time with a median of 7 days, better than Bioplasenton (8 days), Betadine (9 days), 15% extract (11 days), 10% extract (12 days), and negative control (14 days). Histopathological analysis supported these findings, with the 20% group showing optimal reepithelialization, high collagenization activity, no necrosis, and good tissue remodeling. The 20% Java tamarind leaf extract ointment was proven to be the most effective in accelerating wound healing in female white mice compared to lower concentrations or controls. These results support the potential of Java tamarind leaves as a complementary herbal therapy, although clinical trials in humans are still needed to confirm its safety and efficacy

Keywords: Herbal ointment, Mouse model, Re-Epithelialization, *Tamarindus indica*, Wound healing

Introduction

A wound is a physical injury that disrupts tissue integrity in the skin or underlying tissue, thus impeding normal physiological function and anatomical structure. Wounds can occur due to various factors, such as sharp trauma, thermal, chemical, infection, blood vessel disorders, and biological causes, such as animal bites. One common type of open wound is a cut, which is characterised by a linear tear with precise edges and usually without bruising, often found in everyday injuries and surgical procedures. Wound characteristics such as location, shape, size, and depth are significantly associated with the risk of infection, so assessing wound characteristics is crucial in determining treatment and preventing complications [1–3].

Perineal lacerations are a common occurrence during vaginal delivery, whether spontaneous or through an

episiotomy. Perineal injuries can range from mild to severe lacerations affecting the anal sphincter and mucosa. If not managed properly, they can lead to complications such as persistent pain, pelvic floor dysfunction, urinary and faecal incontinence, and sexual dysfunction that can persist long after delivery. The risk of local infection and spread to surrounding organs, such as the bladder and genital tract, also increases if perineal wound management is not optimal. Proper management, from diagnosis, appropriate suturing techniques and materials, to postpartum monitoring, is crucial to accelerate the healing process and reduce the risk of infection and long-term complications [4–6].

Physiological wound healing consists of several main stages: hemostasis, inflammation, proliferation, and maturation/remodelling. This process involves complex interactions between various cell types, chemical mediators, cytokines, and growth factors that play a crucial role in tissue regeneration and

restoration of skin function. Various biological and chemical factors, such as oxygenation, nutritional status, infection, age, hormones, stress, and chronic diseases, can influence the effectiveness of each stage of wound healing. In clinical practice, using ointments or synthetic drugs remains the primary choice to accelerate wound healing. However, long-term use of these drugs can pose a risk of side effects, such as allergic reactions, impaired healing, and potential antibiotic resistance if not used rationally [7–10].

Various herbal plants have been studied as wound healing agents, including aloe vera, bitter melon, betel, African, and binahong. These plants contain active compounds such as flavonoids, alkaloids, saponins, tannins, and phenolics, which are known to have anti-inflammatory, antibacterial, and antioxidant properties. These compounds play a role in accelerating the wound healing process by stimulating cell proliferation, collagen synthesis, and increasing angiogenesis, as well as inhibiting infection and inflammation. The use of herbal plants as wound therapy is considered safer for long-term use because it has minimal side effects compared to synthetic drugs, and can be an effective alternative in wound treatment, both traditional and modern [11–14,38].

The tamarind plant is widely known and used by Indonesians, particularly on the island of Java, as a kitchen spice and ingredient in traditional medicine. In addition to its use in foods such as sour vegetables and herbal medicines, tamarind leaves are used empirically to treat various diseases, including diarrhoea, dysentery, and wounds. Studies have shown tamarind leaves contain bioactive compounds such as flavonoids, tannins, saponins, lupeol, and other metabolites with anti-inflammatory, antibacterial, and antioxidant properties. These compounds have been shown to inhibit microbial growth and increase the migration and proliferation of fibroblasts in wounds, thereby accelerating the tissue healing process [15–17,37].

According to global data, perineal rupture after vaginal delivery is a common complication, with approximately 90% of women experiencing perineal trauma in various countries, including the UK and Germany. In low- and middle-income countries, such as many African countries, the incidence of perineal trauma is also high, with the prevalence of episiotomy

reaching 46% and second-degree rupture at 24%. In Indonesia, similar data were also found, 75% of mothers who gave birth vaginally experienced perineal rupture, with a higher incidence in young age groups and primiparas [18–20].

Materials and Methods

Research design and sampling

This is an actual experimental study with a post-test only control group design. This design was chosen because the experimental animal (*Rattus norvegicus*) allows strict control of variables and direct observation of treatment effects without being influenced by initial measurements. The study subjects were 30 female white rats (*Rattus norvegicus*) that had been acclimatised for 14 days in a laboratory environment. The rats were randomly divided into six groups, each group consisting of 5 rats. The six groups included a negative control group, a positive control group I given Betadine ointment, a positive control group II given Bioplacenton ointment, and three treatment groups, each given tamarind leaf extract ointment with concentrations of 10%, 15%, and 20%.

The study was conducted in the Phytochemistry Laboratory, Pharmacy Laboratory, and Pharmacology Toxicology Laboratory of the Faculty of Pharmacy, Hasanuddin University, Makassar. The Health Research Ethics Committee of the Faculty of Pharmacy, Hasanuddin University, approved ethical clearance for animal experiments under approval number 854/UN4.17/KP.06.05/2025, protocol number UH012502132. This study was conducted between May and July 2025.

Animal subject

The population consisted of healthy female white rats (*Rattus norvegicus*) aged 8–12 weeks, weighing 120–200 grams. Inclusion criteria included clear eyes, clean fur, active movement, and no anatomical skin abnormalities. Rats showing skin lesions or systemic infections were excluded. Subjects who died during the study were categorized as dropouts. The minimum sample size was determined using the Federer formula $(t-1)(n-1) \geq 15$, resulting in five animals per group after adjusting for a potential dropout rate of 15%. Sampling was carried out using

simple random sampling.

Preparation of *Tamarindus indica* L. leaf extract

Fresh *Tamarindus indica* leaves were washed and dried at 40-50°C, then ground into powder. Extraction was done using the Soxhlet method with 70% ethanol as a solvent. The filtrate was concentrated using a water bath at 50°C until a thick extract was obtained. The extract was stored in a dark bottle in cool and dry conditions. Topical ointments were prepared in three concentrations: 10%, 15%, and 20% *Tamarindus indica* L. extract. The ointment was applied to the incision wounds of rats daily for 14 consecutive days. Phytochemical screening of the ethanolic leaf extract of *Tamarindus indica* L. revealed the presence of several secondary metabolites, including tannins, alkaloids, flavonoids, triterpenoids, and saponins.

Intervention procedure

After a 14-day acclimatization period, the mice were anaesthetized using a combination of ketamine and xylazine. A standard incision of $\pm 1 \text{ cm}^2$ was made on the back of each mouse. Each group received its own treatment daily: clean dry wound treatment only group, Betadine ointment group, Bioplacenton ointment group, *Tamarindus indica* extract ointment group 10%, *Tamarindus indica* extract ointment group 15%, and *Tamarindus indica* extract ointment group 20%. Wound healing was assessed using the REEDA (Redness, Oedema, Ecchymosis, Discharge, Approximation) scale, developed by Davidson (1998), with daily observations.

Histological analysis

On day 14, animals were euthanised, and skin samples were harvested and fixed in 10% formalin for 24–48 hours. The tissue underwent standard histological processing: dehydration with graded ethanol, clearing with xylene, and embedding in paraffin. Sections 4–6 μm thick were stained with hematoxylin-eosin and examined under a light microscope (Olympus BX41) at 100x and 400x magnification.

Re-epithelialization thickness was measured at five points on each side of the wound using ImageJ software, and the average value was calculated.

Calibration was performed by adjusting the scale against an embedded micrometre in the histological image.

Statistical analysis

All data were processed using SPSS version 24.0. Editing, coding, data entry, and cleaning were performed before analysis. Univariate analysis described the characteristics of each variable using descriptive statistics. Normality was tested using the Shapiro-Wilk test ($p > 0.05$ indicates a normal distribution).

The Kruskal-Wallis test was used to compare the mean wound healing duration between groups. Post-hoc analysis was applied to determine significant pairwise differences. Linear regression examined the relationship between extract Concentration and wound healing rate.

Ethical considerations

This study adhered to ethical principles in animal experimentation. The research protocol adhered to the 3R principle—Replacement, Reduction, and Refinement—as recommended for animal welfare. Animals were housed in ventilated cages with bedding, provided ad libitum access to water and food, and handled according to humane standards to minimise pain and distress during the study.

Results

This study used female white rats (*Rattus norvegicus*) as test animals, adapted to the environment for 14 days, and divided into six groups, each consisting of 5 female white rats. The results showed that the 20% tamarind leaf extract ointment had the highest effectiveness in accelerating wound healing, with an average epithelialization time of 7 days, faster than bioplasenton (8 days), Betadine (9 days), and concentrations of 15% (11 days) and 10% (12 days). The base ointment without active ingredients had the slowest healing time, at 14 days. These results indicate a positive relationship between increased concentrations of tamarind leaf extract and wound healing speed (Table 1). Analysis of 20% tamarind leaf extract showed the fastest healing results, with a median of only 7 days. This indicates that the higher the concentration of

tamarind leaf extract, the faster the wound healing process. Bioplasenton and Betadine showed medians of 8 and 9 days, respectively, which are still relatively fast but slightly slower than the 20% tamarind leaf extract. Tamarind leaves at 15% and 10% showed medians of 11 and 12 days. This indicates a still good effect, but not as effective as the 20% concentration. The base ointment group (negative control) had the longest median, at 14 days, indicating no significant healing effect without active ingredients. In conclusion, tamarind leaf extract, especially at a concentration of 20%, is more effective in accelerating wound healing compared to the base ointment and even compared to standard drugs such as betadine and bioplasenton, as seen from the median healing time values.

Table 1. Median distribution of wound healing time based on ointment treatment group and female white mice

Treatment	n	Healing Time	Median
Tamarind leaves 10%	5	13,12,13,12,13	12
Tamarind leaves 15%	5	11,12,11,12,11	11
Tamarind leaves 20%	5	7,8,7,8,8	7
Basic ointment	5	14,15,15,14,14	14
Betadine	5	10,9,9,9,11	9
Bioplasenton	5	9,8,9,8,8	8
Total	30		

Table 2. Normality test

Groups	Df	Statistics	Sig	Information
Tamarind leaves 10%	5	.684	.006	Abnormal
Tamarind leaves 15%	5	.684	.006	Abnormal
Tamarind leaves 20%	5	.684	.006	Abnormal
Basic ointment	5	.684	.006	Abnormal
Betadine	5	.684	.006	Abnormal
Bioplasenton	5	.684	.006	Abnormal

Table 2 shows the normality test conducted to determine whether the wound healing time data in each treatment group is normally distributed. Based on the results of the Shapiro-Wilk test, all groups had a significance value (Sig.) < 0.05, ranging from 0.006 to 0.046. This indicates that all data were not normally

distributed. Conclusion: Because the data were not normal, the analysis was continued with non-parametric tests, namely Kruskal-Wallis and Mann-Whitney.

Table 3 shows that there are statistically significant differences between the groups compared. Based on the results of the Kruskal-Wallis test, a p-value of 0.000 was obtained, which is less than 0.05, indicating that there is a very significant difference in wound healing effectiveness between treatment groups (10%, 15%, and 20% tamarind leaves, base ointment, Betadine, and Bioplasenton).

Table 3. Kruskal Wallis test

Groups	n	Mean Rank	p-value*
Tamarind leaves 10%	5	22,60	0,000
Tamarind leaves 15%	5	18,10	
Tamarind leaves 20%	5	3,90	
Basic ointment	5	28,00	
Betadine	5	12,70	
Bioplasenton	5	7,70	

* Kruskal Wallis test

Table 4 shows that 10%, 15%, and 20% tamarind leaf extract (EDA) showed significant differences compared to most of the control groups (basic ointment, betadine, bioplasenton), especially 20% tamarind leaf extract, which showed higher effects in several comparisons. Tamarind leaf 10% had significantly different effectiveness compared to: Tamarind leaf 15% and 20%, basic ointment, Betadine, and Bioplasenton. Tamarind leaf 15%: Significantly different from tamarind leaf 20%, basic ointment, and Bioplasenton. Not significantly different from Betadine ($p = 0.160$), meaning its effectiveness in wound healing is relatively similar to Betadine. 20% tamarind leaves showed higher effectiveness compared to basic ointment and Betadine (significantly different). Not significantly different from Bioplasenton ($p = 0.580$), indicating that both have relatively equivalent wound healing effects.

Table 4. Mann-Whitney test (comparison)

Variables	Average Rating A	Average Rating B	p-value*
Tamarind Leaves 10% Tamarind Leaves 15%	7.60	3.40	0.020
Tamarind Leaves 10% Tamarind Leaves 20%	8.00	3.00	0.007

Tamarind Leaf 10% Base Ointment	3.00	8.00	0.007
Tamarind Leaves 10% Betadine	8.00	3.00	0.007
Tamarind Leaves 10% Bioplaston	8.00	3.00	0.007
Tamarind leaves 15% Tamarind leaves 20%	8.00	3.00	0.007
Tamarind Leaves 15% Base Ointment	3.00	8.00	0.007
Tamarind leaves 15% Betadine	7.70	3.30	0.016
Tamarind leaves 15% Bioplaston	8.0	3.00	0.007
Tamarind Leaves 20% Base Ointment	3.00	8.00	0.007
Tamarind leaves 20% Betadine	3.00	8.00	0.007
Tamarind leaves 20% Bioplaston	3.00	8.00	0.007
Basic Ointment Betadine	8.00	3.00	0.007
Base Ointment Bioplaston	8.00	3.00	0.07
Betadine Bioplaston	5.70	5.30	0.811

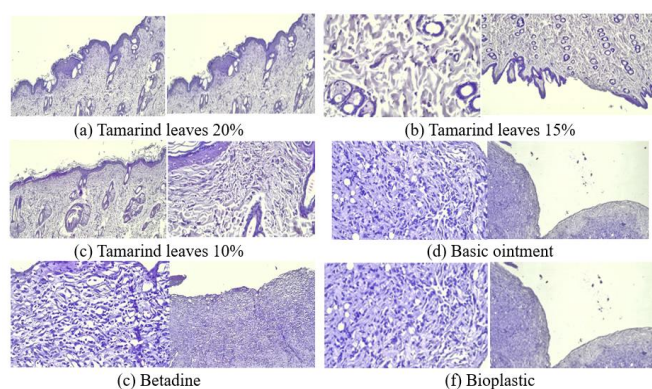
* Mann-Whitney test

Basic Ointment, Betadine, and Bioplasenton are significantly different from one another, indicating that their healing effects vary, with basic ointment tending to be the least effective. A 20% Java tamarind leaf extract is the most effective formulation in accelerating wound healing compared to 10%, 15% extracts, basic ointment, and Betadine, as it shows significant differences in many comparisons. 15% tamarind leaf extract has efficacy comparable to Betadine, as there is no significant difference between the two ($p = 0.160$). Basic ointment has the lowest efficacy, as it is significantly lower than almost all other groups. Bioplasenton and 20% tamarind leaf extract have equivalent efficacy in wound healing (no significant difference).

Based on histopathological results, the 20% tamarind leaf extract group showed the best wound healing

with re-epithelialization, mature collagenization, and tissue resembling normal skin. Bioplasenton was also highly effective, although granulation and angiogenesis were minimal. The 10% extract showed promising results with almost complete healing, while the 15% extract was quite effective, but re-

epithelialization was incomplete. Betadine showed partial healing with severe inflammation and no remodeling. Meanwhile, the base ointment showed the slowest healing, with low epithelialization, moderate inflammation, and no signs of remodeling.



Discussion

The study results showed that the group of mice given 20% tamarind leaf extract ointment experienced the fastest wound healing time, with an average re-epithelialization time of 7 days. This indicates a significant effect of the bioactive content

in tamarind leaf extract on accelerating wound tissue regeneration, especially through proliferation and new tissue formation. A study by Aly et al. (2022) found that tamarind leaf extract also has anti-inflammatory activity and accelerates fibroblast migration, which plays an important role in the wound healing process [22].

Bioactive compounds in tamarind leaf extract, including flavonoids, tannins, saponins, and triterpenoids, play an important role in wound healing. Flavonoids are known to have strong antioxidant and anti-inflammatory activities, as well as supporting epithelial cell regeneration and extracellular matrix formation [23]. Tannins function as astringents, accelerating wound contraction and stopping exudation, while saponins are antimicrobial and accelerate granulation tissue formation [24; 25]. Triterpenoids, such as lupeol, which is found predominantly in tamarind leaf extract, play a role in stimulating epithelialization and inhibiting inflammation [22]. The synergy of these compounds strengthens the pharmacological effects of tamarind leaf extract, which is reflected in the acceleration of the proliferation phase and tissue remodelling in animal models treated with this extract [23,38].

This study found significant differences in wound healing effectiveness between groups ($p < 0.05$), especially between the 20% extract group and positive control groups such as Betadine and Bioplacenton. The results of the Mann-Whitney post-hoc analysis showed that almost all combinations between groups showed significant differences, except between Betadine and Bioplacenton, which had relatively equal effectiveness. However, the 20% extract remained superior with the lowest mean rank (3.90), indicating the fastest wound healing process efficiency among all groups. This finding aligns with the principle that the higher the extract concentration, the higher the wound healing effectiveness. A study by Poernomo et al. (2025) Found that basil leaf extract gel with concentrations of 80% and 90% was more effective in accelerating wound healing than lower concentrations [26]. Other studies also reported that 15% roselle leaf extract [27], 20% mango seed extract [28], and 15% garlic extract [29] Provided more optimal wound healing results than lower concentrations. These findings strengthen the positive relationship between increasing extract concentration and wound healing

effectiveness.

These findings also support the results of previous studies by Goher et al. (2024) and Aly et al. (2022), which showed that *Tamarindus indica* extract can accelerate the epithelialization process and new tissue formation. In the study by Goher et al. (2024) The use of tamarind leaf extract in the form of topical nanofibers significantly increased wound healing activity, both in vitro and in vivo, and accelerated fibroblast migration and tissue re-epithelialization [23]. Meanwhile, Aly et al. (2022) Reported that n-hexane extracts from various *T. indica* organs, including leaves, effectively increased fibroblast migration and accelerated wound closure in in vitro tests, supported by anti-inflammatory activity and histopathological observations [22]. Tamarind leaf extract has the potential to be developed as an effective wound healing agent with minimal risk of side effects.

The histopathological results of these findings indicate that the 20% treatment group showed the most optimal results, with maximum scores in re-epithelialization and tissue remodelling, without necrosis or inflammation. In contrast, the control groups, such as basic ointment and Betadine, showed minimal re-epithelialization, accompanied by moderate inflammation and necrosis. This indicates that although antiseptics such as Betadine effectively prevent infection, their long-term use can inhibit cell regeneration due to tissue irritation. These findings are consistent with reports by Ortega-Llamas et al. (2022) and Kanjevac et al. (2017), which state that some antiseptics are cytotoxic to developing new epithelial cells [30,31].

The effectiveness of tamarind leaf extract can also be attributed to its antimicrobial activity, which contributes to preventing secondary wound infections. Infection is a major factor inhibiting wound healing because it triggers prolonged inflammation and degradation of the extracellular matrix. Studies by Aminu et al. (2025) and Zheng et al. (2021) showed that tamarind leaf extract can inhibit the growth of various pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, which are often found in open wounds [32,33]. By reducing the microbial load in the wound area, the healing process can proceed more optimally without excessive inflammation. This explains why the group

given 20% extract showed faster re-epithelialization compared to other control groups, including antiseptic ointments that do not have direct regenerative effects [34].

The Bioplacenton group showed few signs of mild inflammation on microscopic examination, despite reaching the remodelling phase. This indicates that tamarind leaf extract at a concentration of 20% accelerated healing and significantly reduced inflammation level compared to existing commercial therapies. Applying *tamarindus indica* extract also did not cause inflammation, as sometimes occurs with synthetic agents, and its wound healing effectiveness was at least equal to or even better than commercial agents such as Solcoseryl or silver sulfadiazine. The extract is an alternative that not only accelerates recovery but also reduces the risk of inflammatory complications in wounds [35].

Individual variability between experimental animals, such as nutritional status and body weight, can affect wound healing. Mice with lower body weight tend to experience slower healing, which is one of the limitations of this study. A study by Wang et al. (2022) Stated that mice with good nutritional status or a balanced diet showed a faster healing process [36, 39]. Findings by Seth et al. (2024) Also reported that mice with lower body weight or poor nutritional status can also experience impaired healing due to limited energy and protein reserves for the tissue regeneration process [37].

Conclusion

Tamarind (*Tamarindus indica* L.) leaf extract ointment significantly accelerated the wound healing, particularly at a 20% concentration, demonstrating the best results in epithelialization and tissue remodelling. The extract's active compounds, such as flavonoids and tannins, significantly contribute to its anti-inflammatory and regenerative activities. For further development, this study can serve as a basis for broader herbal-based topical formulations, including human clinical trials and exploration of the ointment formulation's long-term stability and safety.

Ethical considerations

The study protocol was reviewed and approved by

the Health Research Ethics Committee of the Faculty of Pharmacy, Hasanuddin University, with approval number 854/UN4.17/KP.06.05/2025. All procedures adhered to animal welfare principles and the 3Rs (Replacement, Reduction, and Improvement). No plagiarism, data fabrication, or redundancy occurred. All data and findings were reported transparently and accurately.

Conflict of interest

The author declares that there is no conflict of interest.

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