

Test the Effectiveness of Tegari Leaf Extract (*Dianella nemorosa* Lam.) from Papua on Burn Wound Healing in Rabbit (*Oryctolagus cuniculus*): Experimental Study

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ABSTRACT

The tegari plant (*Dianella nemorosa* Lam.) known locally as "pra kepey" (Papua). *D. nemorosa* Lam. plant is one of the traditional medicines from Papua which is used as a medicine to heal external wounds such as cuts, burns, and internal wounds. The aim of this research was to test the effectiveness of tegari leaf extract and to test the concentration of tegari leaf extract from Papua which was used most effectively for healing burns in rabbits. Each rabbit's back was divided into 6 control treatments, that is normal control (no treatment), negative control (Vaseline album), positive control (conventional burn gel), and *D. nemorosa* Lam. extract with a concentration of 0.1%, 0.5%, and 1%. The rabbit's back was anesthetized using 2% lidocaine HCl subcutaneously and induced with an iron metal diameter of 2.2 cm and a thickness of 1 mm which was heated in a blue flame for 3 minutes and then placed on the rabbit's back for 5 seconds until a second degree (deep) burn was formed. The diameter of the rabbit's back burn wound was measured for 21 days. The results of the percentage of healing of burn wounds from *D. nemorosa* Lam. extract at concentrations of 0.1%, 0.5%, and 1%, respectively, were 76.80%, 79.00%, and 87.40% on day 21st. The data were analyzed using one-way ANOVA, namely in the form of the percentage of burn wound healing which showed that there were differences in the effect of burn wound healing in each control treatment group because the significant value was $p < 0,00$ ($p < 0,05$) indicating that there were significant differences between each group. *D. nemorosa* Lam. extract is effective in healing burns.

KEYWORDS: Effectiveness; *Dianella nemorosa* Lam.; Leaf; Extract; Burns; Healing; Rabbit.

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I. INTRODUCTION

The traditional Papuan plant Tegari (*D. nemorosa* Lam), locally called "Pra Kepey" belongs to the Liliaceae family. The people of Tablanus Depapre, Papua, have been utilizing tegari plants as traditional medicine [18]. Several plant species in the Liliaceae family are known to have various pharmacological effects, including anticancer, antioxidant, immunomodulatory, and antibacterial effects [1, 4, 6, 12, 33].

Burns are wounds caused by the direct or indirect contact of the body's surface with heat-producing items [3].

Burns are traumatic injuries produced by being exposed to chemicals, fire, radiation, or electricity. The transmission of energy from a heat source to the human body can have physiological consequences, including irreparable tissue damage [19].

The alkaloid compounds behave as antimicrobials to disrupt the various parts of peptidoglycan in bacterial cells so that the cell wall layer remains intact and causes the death of cells [24], saponin compounds stimulate collagen formation, which plays a role in increasing tissue

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epithelialization so that it can close the wound surface [28], and flavonoid compounds work as antioxidants by inhibiting the process of lipid peroxidation, which increases collagen Tannin chemicals act as an astringent. Tannins work as an astringent, closing skin pores and preventing exudate and bleeding to seal wounds [16]. Herbal treatments are often safer and have fewer adverse effects [25]. Scientists have turned their attention to the potential healing characteristics of plants with medicinal values, and there have been several reports on the use of herbal medicines to treat skin wounds; the cost difference between herbal medicines and synthetic medicines is significant, which has increased interest in their use [8, 9].

II. MATERIAL AND METHODS

A. Plant Materials And Extraction

Tegari plant (*D. nemorosa* Lam.) was obtained in Tablanusu Village, Depapre District, Jayapura Regency, Papua. The Herbal Medical Laboratory confirmed the plant as *D. nemorosa* Lam of the Liliaceae family with determination number 000.9.3/789/102.20/2024. The tegari leaves were cleaned first, then washed under running water. afterward, a component of leaves are sliced into small sections and drained. Then it is dried in an oven at 50°C until dry. After drying, the tegari plant is blended and filtered using a mesh sieve no. 100 to produce a fine powder of tegari leaves, which is then kept in a tightly covered container. The powder was extracted using maceration with ethanol. The extract was centralized with a water bath. resulting thick extract is then calculated as % yield against the weight of the simplisia using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of extracted simplisia powder (g)}} \times 100\%$$

B. Tools

The tools used are laboratory glassware, porcelain cup, blender, glass jar, analytical balance, mesh no. 100, rotary evaporator, water bath, vernier caliper, shaver, mortar and stemper, hand scoop, aluminum foil, disposable syringe three cc, iron plate diameter 2.2 cm with a thickness of 1 mm, ointment pot, and rabbit animal cage.

C. Materials

The materials used in this study were tegari leaves (*D. nemorosa* Lam.), 96% ethanol, vaseline album, distilled water, lidocaine HCl 2%, K₂Cr₂O₇, H₂SO₄, HCl, FeCl₃, C₄H₆O₃, amyl alcohol, Magnesium powder, Mayer reagent, Dragendroff reagent, conventional gel, filter paper, and rabbit feed.

D. Animal Studies

This study's test animals included five male rabbits (*Oryctolagus cuniculus*) weighing 1.5 kg, 1.6 kg, 1.9 kg, 2 kg, and 2.1 kg. This study was carried out with approval from the Muhammadiyah University of Banjarmasin's

Health Research Ethics Commission, which issued a certificate of research feasibility with the KEPK number 0128226371. Rabbit test animals (*Oryctolagus cuniculus*) are acclimatized first so that they can adjust to the ambient conditions that will be used in the study. Test animals are acclimated by keeping them indoors for five days at normal temperature and feeding them standard foods and beverages in moderation. Rabbit test animals can be deemed to be healthy if, throughout acclimation, rabbits do not exhibit changes in body weight. Physically visible rabbits, there are no symptoms of the disease.

E. Create An Acute Wound Mode

In the test animals the fur on the rabbit's back was shaved 240 cm² or (12×20 cm), measured using a ruler, and the back area was divided into six wound areas. Before treatment, wash hands first, use sterile gloves, and perform aseptic measures in the shaved skin area using 70% alcohol. Then, anesthetize the rabbit's skin using a 2% lidocaine ampoule. Then, burns on the rabbit's back with a modified tool made of an iron plate with a diameter of 2.2 cm and a thickness of 1 mm, which is heated in a blue flame for 3 minutes before being connected to the rabbit's back for 5 seconds until a second-degree burn (deep) is generated [14].

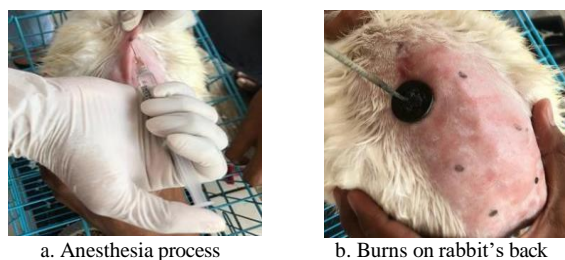
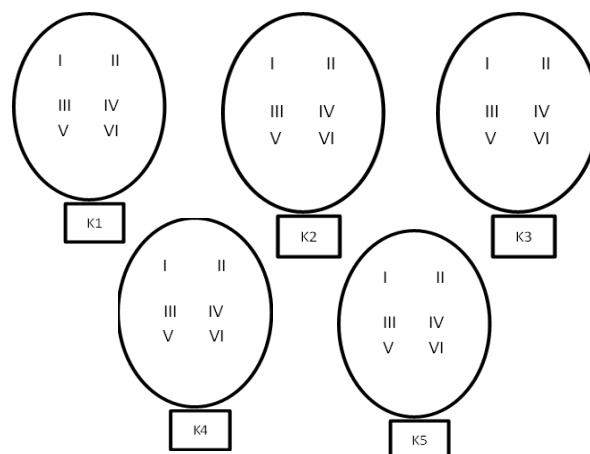


Figure 1. a. Anesthesia process using lidocaine HCl 2%, b. Burn wound creation

F. Studied Groups

Figure 2 shows how each rabbit's six wound locations will be treated individually. The burns were treated with varying doses of tegari leaf extract (*D. nemorosa* Lam.) in vaseline album carrier, conventional gel as a positive control, and vaseline album as a negative control, twice daily in the morning and evening at 0.3 g for 21 days



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Description:

- I : No treatment (normal control)
- II : Administered vaseline album (negative control)
- III : Administered conventional burn gel (positive control)
- IV : Given tegari leaf extract (*D. nemorosa* Lam.) concentration of 0.1% in vaseline
- V : Given tegari leaf extract (*D. nemorosa* Lam.) concentration of 0.5% in vaseline
- VI : Given tegari leaf extract (*D. nemorosa* Lam.) concentration of 1% in vaseline
- K1 : First rabbit test animal
- K2 : Second rabbit test animal
- K3 : Third rabbit test animal
- K4 : Fourth rabbit test animal
- K5 : Fifth rabbit test animal

Figure 2. Model of animal treatment

G. Wound Healing Rate Determination

The Morton technique requires fixedly measuring four wound sizes using a caliper until the wound diameter is zero. The average diameter each day is then determined. Every day, test animals' burns were inspected, and every three days that is, on days 1, 3, 6, 9, 12, 15, 18, and 21, diameter measurements were obtained. The following formula determined the burn wound's average diameter:

$$dx = \frac{d1 + d2 + d3 + d4}{4}$$

Description:

dx: average burn diameter of each treatment replicated

$d_{(1,2,3,4)}$: diameter of each part

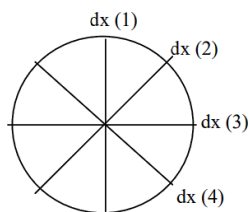


Figure 3. Average Burn Diameter Measurement

[10] Healing percentage formula:

$$p\% = \frac{do - dx}{do} \times 100\%$$

Description:

P%: Percentage of wound healing (%)

dx: Average burn wound diameter (mm)

do: Initial wound diameter (mm)

H. Data Analysis

All groups' quantitative data were evaluated using SPSS version 15 software. $P < 0.05$ was deemed significant. All data were presented using the One-Way ANOVA test, and Duncan's significant differences between groups were examined.

III. RESULTS AND DISCUSSION

A. Tegari Leaf Extraction Results

Tegari Leaf Extraction Results The results of extraction by maceration method from tegari leaf simplisia (*D. nemorosa* Lam.) are as much as 200 g using 96% ethanol as much as 3000 mL, which is obtained as much as 22.60 g thick extract with % yield obtained by 11.30%.

Table 1. Tegari Leaf Extraction Results

| Sampel | Simplicia Weight (g) | Solvent Vloume (mL) | Extract Weight (g) | Yield (% b/v) |
|-------------------------|----------------------|---------------------|--------------------|---------------|
| <i>D. nemorosa</i> Lam. | 200 | 3000 | 22,60 | 11.30 |

B. Phytochemical Screening Results

Phytochemical screening is a particular technique for determining the amounts of secondary metabolite chemicals in natural substances. Based on the findings of phytochemical screening shown in table 2.2, tegari leaf extract (*D. nemorosa* Lam.) from Tablanusu Village, Depapre District, Jayapura Regency, provides alkaloid compounds, saponins, flavonoids, and tannins.

Table 2. Phytochemical Screening Results

| Sample Test | Compounds | Extrac t | Descriptions |
|--------------------------|---------------|----------|--|
| <i>D. nemorosa</i> Lam.) | Alkaloids | + | Formed orange precipitate (Mayer) and formed a white precipitate (Dragendroff) |
| | Flavonoids | + | Formed orange to red color |
| | Saponins | + | Form of stable foam. |
| | Tannins | + | Blackish green color |
| | Steroids | - | Formed bluish-green color |
| | Triterpenoids | - | Formed brown or violet rings |

Description:

(+) = Detected containing compounds

(-) = Not detected containing compounds

C. Burn Healing Effectiveness Test

Summary the burns observed in this study were

second-degree (deep) burns that caused damage to the epidermis and a portion of the dermis, which is an acute inflammatory reaction characterized by exudation, blistering, a red or pale wound base that is higher than the normal skin surface, and pain caused by irritated nerve ends. Second-degree (deep) burns cause damage to nearly every portion of the dermis. Skin appendages, including hair follicles, sweat glands, and sebaceous glands, are partly intact [26].

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Table 3. Mean Percentage of Burn Wound Healing

| Treatment Groups | Mean percentage of burn wound healing D-21 (%) ± SD |
|---------------------|---|
| Normal control | 56.20 ± 5.54 |
| Negative control | 72.60 ± 6.39 |
| Positive control | 82.40 ± 6.43 |
| 0,1 % Concentration | 76.80 ± 6.87 |
| 0,5% Concentration | 79.00 ± 7.31 |
| 1% Concentration | 87.40 ± 7.89 |

According to Table 3, the average percentage of burn wound healing is 87.40% in the 1% concentration treatment group and 82.40% in the positive control treatment group. These two groups have the highest percentages of burn wound healing. The group receiving normal treatment had the lowest percentage of burn wound healing (56.20%). Studies by Dias et al. (2009) and Nhung et al. (2019) demonstrated that Australian medicinal plants belonging to the *Liliaceae* family, more specifically *Dianella callicarpa*, released new naphthalene glycosides, dianellose, dianellin, dianellidin, dianellinone, stellalderol, and 5- hydroxydianellin, which have antiviral and antimicrobial activities which assist in wound healing [7, 21].

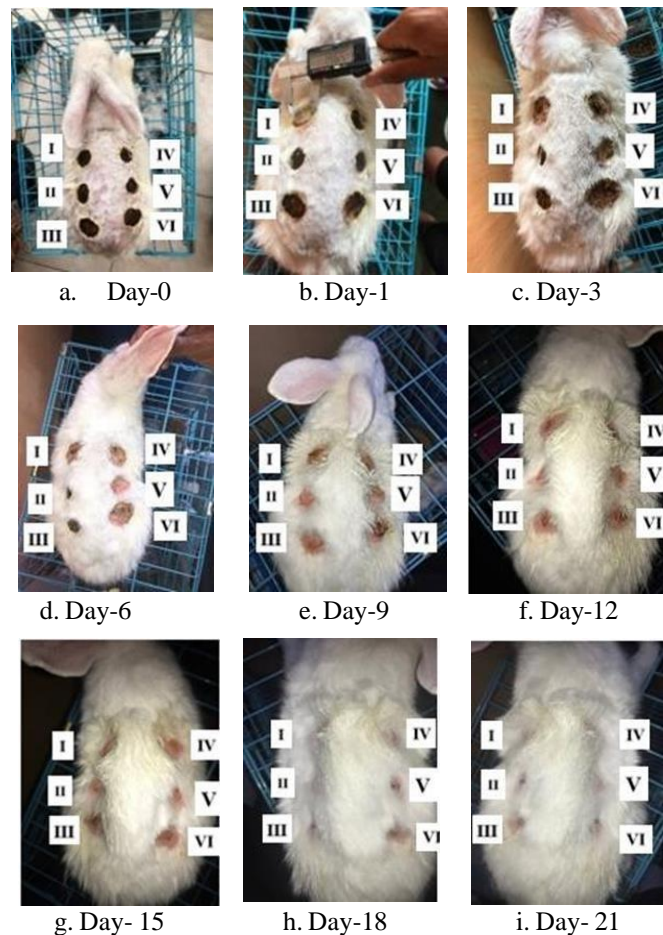


Figure 4. Observation process of burn wound healing in rabbits from H-0 to H-21. I) no treatment (standard control), II) vaseline album (negative control), III) conventional burn gel (positive control), IV) tegari leaf extract (*D. nemorosa* Lam.) 0.1% concentration in vaseline, V) tegari leaf extract (*D. nemorosa* Lam.) 0.5% concentration in vaseline, VI) tegari leaf extract (*D. nemorosa* Lam.) 1% concentration in vaseline.

The typical control group treatment, which receives no therapy, is designed to assess the size of the innate immune system, which consists of several defensive mechanisms that provide main protection against dangerous chemicals during the burn wound healing process [28]. On day 21, the normal control group had a burn wound healing rate of 56.20 percent. The burn wound healing process begins with the inflammatory phase, which lasts from day 0 to day 10; the proliferation phase, which lasts from day 11 to day 28, and the remodeling or maturation phase, which lasts from day 29 to day 31, during which the wound decreases but does not heal completely.

In the negative control group, treatment with only vaseline album resulted in a healing percentage of 72.60%. The burn wound healing process was observed in the inflammatory phase from day 0 to day 7, the proliferation phase from day 8 to day 28, and the wound experienced a remodeling or maturation process from day 29 to day 31. The wound decreased but did not heal completely. Therefore, on the 21st day, the negative control treatment group produced findings that were not significantly distinct from the 0.1% concentration treatment group or the 0.5% concentration treatment group.

Vaseline album, a hydrocarbon carrier, can soften the skin layer (emollient) and enhance skin moisture, allowing it to survive water evaporation in burns [29]. Vaseline album softens and occludes the skin layer, increasing skin hydration by limiting the loss of water [2].

In the positive control group, standard burn gel therapy resulted in an 82.40% healing rate; the positive control treatment group served as a comparison to the other groups' treatments. The burn healing process that was observed during the inflammatory phase lasted only two days. This differs from the 1% concentration therapy, which experienced an inflammatory phase from day 0 to day 3, due to the presence of placenta extract in traditional gel, which acts as an anti-inflammatory and so involves a part in the burn wound healing process. The proliferation phase lasts from day 3 to day 10, and from day 11 to day 21, the wound undergoes a remodeling or maturation process; the wound is closed but has not yet experienced complete healing.

Conventional gels include two ingredients: placenta extract and neomycin. Placenta extract has anti- inflammatory and analgesic properties and regulates TGF-β, which regulates fibroblast cell proliferation and the extracellular matrix [20, 24], as well as VEGF, which plays a role in angiogenesis [15]. Neomycin is a class of

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aminoglycoside antibiotics that limit bacterial protein synthesis, resulting in bactericidal activity.

In the 0.5% concentration group given tegari leaf extract (*D. nemorosa* Lam.) in vaseline carrier showed a healing percentage of 79.00%, the burn wound healing process observed in the inflammatory phase lasted from day 0 to day 4, and the proliferation phase occurred on day 5 to day 10, the wound received a remodeling or maturation process on day 11 to day 21, the wound was closed but had not yet experienced total healing.

Tegari leaf extract (*D. nemorosa* Lam.) in vaseline carrier exhibited an 87.40% healing rate in the 1% concentration group therapy. The burn wound healing process observed in the inflammatory phase lasts from day 0 to day 3; this differs from the concentrations of 0.1% and 0.5% because the higher the concentration of tegari leaf extract used, the greater the content of secondary metabolite compounds that can provide anti-inflammatory activity [22]. The proliferation phase lasts from day 4 to day 8, and from day 9 to day 21, the wound undergoes remodeling or maturation; it is closed but not completely healed.

Alkaloid compounds found in Tegari leaf extract (*D. nemorosa* Lam.) play a crucial function in burn wound healing because they can act as antimicrobials by disrupting the components that build up the peptidoglycan in bacterial cells, causing the cell wall layer to not form intact and resulting in cell death [23]. Flavonoids prevent the development of germs in skin tissue and function as anti-inflammatory agents by decreasing the activity of the enzyme cyclooxygenase [5, 34]. Flavonoid chemicals also act as antioxidants, inhibiting the lipid peroxidation process, which increases collagen fibers, protects cells, and promotes DNA production [31].

Saponin compounds stimulate collagen synthesis, which helps to increase tissue epithelialization and shut the burning surface [27]. Tannin compounds discovered in Tegari leaf extract (*D. nemorosa* Lam.) have a crucial function in burn healing because they serve as an astringent. Tannins work as astringents, closing skin pores and preventing exudate and bleeding to help heal the wound [16].

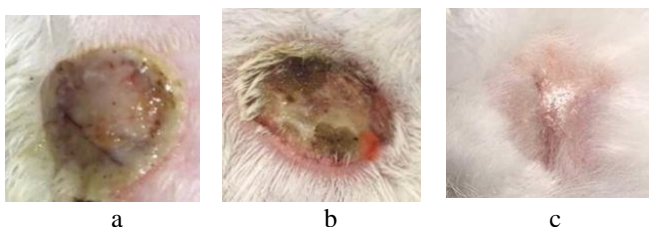


Figure 5. a. Observation of Inflammatory Phase, b. Proliferation Phase, and c. Remodeling Phase

According to Swift et al. (1999), characteristics that may assist with wound healing include age, delays in re-epithelialization, collagen production, and angiogenesis, all of which have been found in aged rats. Compared with

young rats [30]. The hormone estrogen influences wound healing by modulating genetics involved in regeneration, matrix synthesis, protease inhibition, epidermal function, and inflammation [13]. According to Johnson & Richard (2003) and Tecklin (2002), acute second-degree burns can take three to six weeks or more to heal without the use of particular therapeutic agents, leaving scar tissue [17, 32].

CONCLUSIONS

Based on the results of this study, it is concluded that the leaf extract of tegari leaves (*D. nemorosa* Lam.) from Papua has effectiveness on burn healing in rabbits (*Oryctolagus cuniculus*). The concentration of tegari leaf extract (*D. nemorosa* Lam.) from Papua that is most effective in healing burns is 0.5%, with a percentage of burn healing of 79.00% on day 21.

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