

EFFECT OF GEL FORMULATION CONTAINING *Tridax procumbens* AND *Aloe vera* LEAF EXTRACT ON THE HEALING OF SECOND-DEGREE BURNS IN MICE (*Mus musculus*)

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Abstract

Tridax procumbens L. and *Aloe vera* L. are plants commonly used in the treatment of burns. This study was conducted to determine the effect of giving a combination gel of *T. procumbens* and *A. vera* leaf extract on second-degree burn healing in mice. This study used 24 male mice (*Mus musculus*) which were divided into four treatment groups, namely positive control (KP, Bioplacenton®); negative control (KN, gel base); leaf extract of *T. procumbens* 0.5% and *A. vera* 0.5% (P1); and leaf extract of *T. procumbens* 1% and *A. vera* 0.5% (P2). The process of inducing burns on the backs of mice requires the use of a 1x1 cm iron, which is subjected to heat in a fire for three minutes. The iron is placed on the mice's back for five seconds, which leads to the development of a second-degree burn. The gel was applied twice a day for 21 days. The highest level of burn wound healing was recorded with the Bioplacenton® treatment, which achieved a full recovery of 100% on day 15. Following this, the combined gel treatment of 1% *T. procumbens* leaf extract and 0.5% *A. vera* also reached 100% healing by day 18. While it may not be as potent as Bioplacenton®, the gel formulation containing 1% *T. procumbens* and 0.5% *A. vera* leaf extract presents a viable alternative for treating second-degree burns.

Key words: *Aloe vera*, Bioplacenton®, second degree burns, *Tridax procumbens*.

INTRODUCTION

A burn is the destruction of skin tissue from contact with heat sources like water, fire, chemicals, electricity, or radiation, potentially damaging skin and affecting body systems (Rahmadani et al., 2021). Burns can compromise skin integrity and have complex systemic effects. They are classified into three degrees: first degree (superficial, affecting the epidermis), second degree (partial thickness, involving the dermis and some subcutaneous tissue), and third degree (full thickness, affecting the entire epidermis, dermis, and subcutaneous tissue). Second-degree burns may cause water-filled blisters or ulcers due to heat, electrical, chemical, or radiation exposure (Anggraini et al., 2019). Research on medicinal plants in Indonesia has shown many with therapeutic properties, offering fewer side effects than synthetic medications (Wardani, 2020).

The plant *Tridax procumbens* L. or songgolangit (Indonesian local name) is noted for its medicinal properties and is rich in flavonoids, anthraquinones, phenolics, saponins, steroids, tannins, and

triterpenoids (Permatasari, 2020). *Aloe vera* L. or lidah buaya (Indonesian local name), thriving in tropical and subtropical regions, has long been valued for its medicinal properties. Comprising 95% water, it also contains amino acids, essential oils, minerals, vitamins, enzymes, and glycoproteins. Chemically, *aloe vera* includes auxins, gibberellins, anthraquinones, and vitamins A, C, and E, and it possesses anti-inflammatory, anticancer, antipyretic, antifungal, antioxidant, antiseptic, antimicrobial, and antiviral properties (Puspitosari et al., 2021).

Traditional medicine has been practiced since ancient times and passed down through generations. However, with scientific advancements, many now prefer chemical medications seen as more effective (Taruh, 2021). Natural ingredients like Aloe Vera (*Aloe vera* L.) are commonly used to treat dry hair, strengthen roots, and reduce hair loss (Masyitoh et al., 2019). This plant is also used for facial skin treatment and serves as both a medicinal resource and a key ingredient in cosmetics (Mulianingsih et al., 2021). Research on a gel combining *T. procumbens* and *A. vera* leaf extract for treating burns has never been conducted before, so researchers aim to investigate the effect of applying a combination gel of these two extracts in aiding the healing process of second-degree burns in mice (*Mus musculus*).

RESEARCH METHODS

This research was conducted from September to November 2024 at the Biology Laboratory, Department of Biology, Faculty of Mathematics, Natural and Earth Sciences, Universitas Negeri Manado, North Sulawesi, Indonesia.

Tridax procumbens L. Leaf Extraction

The extract was prepared from fresh, green leaves of *T. procumbens* collected near Universitas Negeri Manado. Only leaves that were free from hollowness and mold were included, while wilted, yellow, or damaged leaves were excluded. Following Mitasari's (2022) method, 3 kg of suitable leaves were washed, air-dried for 24 hours, and then dried in an oven at 40°C for another 24 hours. The dried leaves were ground into powder and macerated in 70% ethanol for 48 hours. After filtering, the filtrate was concentrated using a rotary evaporator at 50°C to produce a thick extract, which was then dried in an oven at 40°C to form a paste-like consistency and dissolved in a 1% Na-CMC solution.

Aloe vera L. Leaf Extraction

Aloe vera obtained from North Minahasa and Tondano was cleaned, peeled, and processed into 1kg of gel, which was then blended into a pulp. This pulp was subjected to maceration in 1000 mL of 70% ethanol for 48 hours, after which it was filtered to collect the filtrate, which was concentrated using a rotary evaporator (Alishia and Firani, 2019).

Evaluation of Gel Preparation

An organoleptic assessment is conducted to evaluate the physical characteristics of the formulation through direct observations of its shape, color, and aroma. The homogeneity assessment is performed

to ascertain whether the formulation is homogeneous, which is indicated by the lack of coarse particles at ambient temperature (Aryati et al., 2019).

Formulation of Burn Gel Preparations

To formulate the burn gel, Na-CMC was mixed with hot water (60°C) at a ratio of 1:20 and allowed to hydrate for 30 minutes. Meanwhile, nipagin was dissolved in distilled water and blended into the Na-CMC mixture along with glycerin and propylene glycol until a smooth consistency was achieved. Distilled water was then slowly incorporated while stirring with a homogenizer until a gel was formed. Once the mixture was uniform, *A. vera* and *T. procumbens* leaf extracts were dissolved in distilled water and added to the gel, ensuring an even distribution (Novitasari, 2018).

Table 1. Formulation of Gel Mixture of *Tridax procumbens* L. and *Aloe vera* L. Leaf Extract.

Material	Positive Control (KP)	Negative Control (KN)	Treatment 1 (P1)	Treatment 2 (P2)
Na CMC	Bioplacenton®	3%	3%	3%
Glycerin		7.5%	7.5%	7.5%
Propylene glycol		3.75%	3.75%	3.75%
Nipagin		0.05%	0.05%	0.05%
Aqua bidest		60%	60%	60%
<i>T. procumbens</i> Extract		-	0.5%	1%
<i>A. vera</i> Extract		-	0.5%	0.5%

Description: KP = positive control, treated with Bioplacenton®; KN = Negative Control, treated with gel base; P1 = Treatment 1, treated with 0.5% *T. procumbens* and 0.5% *A. vera* leaf extract; P2 = Treatment 2, treated with 1% *T. procumbens* and 0.5% *A. vera* leaf extract.

Animals Treatment

The experiment involved 24 mice (*Mus musculus*), aged 2 to 3 months and weighing between 25 and 30 grams. These mice were divided into four groups, each containing six individuals. Before the treatment commenced, the mice were allowed a one-week acclimatization period during which they had unlimited access to food and water (Elsi et al., 2024).

Creating Burns in Mice

The mice's hair on the area to be injured is shaved first, and then the skin is cleaned with 70% alcohol. A 3-minute heat of 1x1 cm iron is applied to the mice's back for five seconds to induce a second-degree burn. This procedure follows the modified method of Prasongko et al. (2020).

Table 2. Treatment Group Design

Group	Number of Mice	Treatment
I	6	Positive control, mice were treated with commercial gel (Bioplacenton®).
II	6	Negative control, mice were treated with gel base without extracts.
III	6	Mice were treated with a combination of 0.5% <i>T. procumbens</i> and 0.5% <i>A. vera</i> leaf extract.
IV	6	Mice were treated with a combination of 1% <i>T. procumbens</i> and 0.5% <i>A. vera</i> leaf extract.

Anatomical Observations

Anatomical pathology observations were made daily starting from day 1 to day 21 after wounding in all treated mice. Observations were made by looking at the wound. To assess wound healing, photos of mice affected by burns were taken every day, then processed using ImageJ Software, and the percentage of healing was calculated. The percentage of wound healing was determined using the formula:

$$\% \text{ Wound Healing} = \frac{\text{initial wound area} - \text{final wound area}}{\text{initial wound area}} \times 100\%$$

The initial wound area refers to the size of the wound measured on the day following the injury, while the final wound area is assessed on the day of observation. The initial wound area used to determine the percentage of wound healing is specifically the area measured the day after the mice have been wounded, as it is observed that after 24 hours, the wound area stabilizes (Novitasari, 2018).

Data Analysis

Analysis of mice body weight data was carried out using the SPSS for Windows version 22 program with the Homogeneity of variance test to determine whether the data were normally or abnormally distributed. If the data were normally distributed ($p \geq 0.05$), the one-way test and Duncan's post hoc test were performed. Wound healing data were displayed descriptively in the form of tables and figures.

RESULTS AND DISCUSSION

Extraction Results and Evaluation of Gel Preparation

The process of making *T. procumbens* and *A. vera* leaf extract used the maceration method with 70% alcohol solvent for 48 hours. The filtered maceration results were evaporated using a rotary evaporator, producing crude extracts (Table 3). Evaluation of gel preparations of *T. procumbens* and *A. vera* leaf extract included organoleptic tests and gel homogeneity (Table 4).

Table 3. Extraction Results of Treatment Materials

Material (wet weight)	Simplified (dry weight)	Solvent	Crude Extract Yield
<i>A. vera</i> leaf: 1 kg	-	Alcohol 70% 1 L	5.108 g
<i>T. procumbens</i> leaf: 3 kg	104 g	Alcohol 70% 2 L	5.330 g

Table 4. Gel Preparation Evaluation

Characteristics	Treatments			
	KP	KN	P1	P2
Color	Clear transparent	Clear transparent	Transparent light green	Transparent light green
Organoleptic Form	Semisolid transparent	Semisolid transparent	Semisolid transparent	Semisolid transparent
Scent	Typical	Typical	Distinctive, sharper, leaf scent	Distinctive, sharper, leaf scent

Homogeneity	Homogeneous	Homogeneous	There are still grains	There are still grains
Description: KP = positive control, treated with Bioplacenton®; KN = Negative Control, treated with gel base; P1 = Treatment 1, treated with 0.5% <i>T. procumbens</i> and 0.5% <i>A. vera</i> leaf extract; P2 = Treatment 2, treated with 1% <i>T. procumbens</i> and 0.5% <i>A. vera</i> leaf extract.				

Body Weight of Mice

The body weight of mice was observed before and after treatment to determine the condition during the treatment. The initial body weight and final body weight of mice are shown in Table 3.

Table 5. Initial and Final Body Weight of Mice

Treatment	Initial Body Weight	Final Body Weight	Weight Difference
KP	18.50 ± 2.949 a	19.67 ± 3.265 a	1.17 ± 0.752 a
KN	17.33 ± 2.160 a	18.50 ± 2.428 a	1.17 ± 0.983 a
P1	18.17 ± 2.316 a	18.67 ± 2.503 a	0.50 ± 2.073 a
P2	17.17 ± 2.926 a	18.83 ± 2.857 a	1.67 ± 1.366 a

Description: KP = positive control, treated with Bioplacenton®; KN = Negative Control, treated with gel base; P1 = Treatment 1, treated with 0.5% *T. procumbens* and 0.5% *A. vera* leaf extract; P2 = Treatment 2, treated with 1% *T. procumbens* and 0.5% *A. vera* leaf extract.

Percentage of Second-Degree Burns in Mice

Observation of wounds in mice was carried out daily and documented on days 3, 6, 9, 12, 15, 18 and 21. The percentage of second-degree burn area in mice is presented in a histogram (Figure 1).

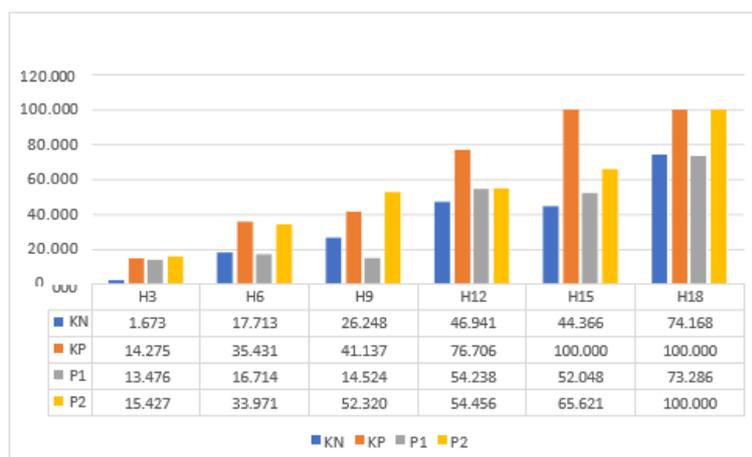


Figure 1. Histogram of second-degree burns in mice in each group.

KP = positive control, treated with Bioplacenton®; KN = Negative Control, treated with gel base; P1 = Treatment 1, treated with 0.5% *T. procumbens* and 0.5% *A. vera* leaf extract; P2 = Treatment 2, treated with 1% *T. procumbens* and 0.5% *A. vera* leaf extract.

On day three, mice treated with Bioplacenton® (KP) and those receiving a gel with *T. procumbens* and *A. vera* leaf extracts (P1 and P2) showed burn wound healing percentages of 13% to 15%. By day six, Bioplacenton® had the highest healing rate at 35.43%, followed by P2 at 33.97%. In contrast, KN (gel

base) and P1 had healing rates of 17.71% and 16.71%, respectively. On day nine, all groups improved, with P2 leading at 52.32%, followed by KP at 41.13%, KN at 26.24%, and P1 at 14.52%. By day twelve, KP reached 76.7%, the highest healing percentage, while P2 had 54.4%, P1 had 54.2%, and KN had the lowest at 46.9%. On day fifteen, the Bioplacenton® treatment group (KP) showed complete healing of burns. In contrast, the 1% *T. procumbens* and 0.5% *A. vera* leaf extract group (P2) had a healing rate of 65.6%, while the 0.5% *T. procumbens* and 0.5% *A. vera* leaf extract group recorded 52%. The negative control group (KN) had a healing percentage of 44.3%. By day eighteen, the P2 group had fully healed burn wounds. The gel base group (KN) achieved a healing rate of 74.1%, while the P1 group with 0.5% *T. procumbens* and 0.5% *A. vera* leaf extract recorded 52%. By day twenty-one, all mice had completely recovered from second-degree burns.

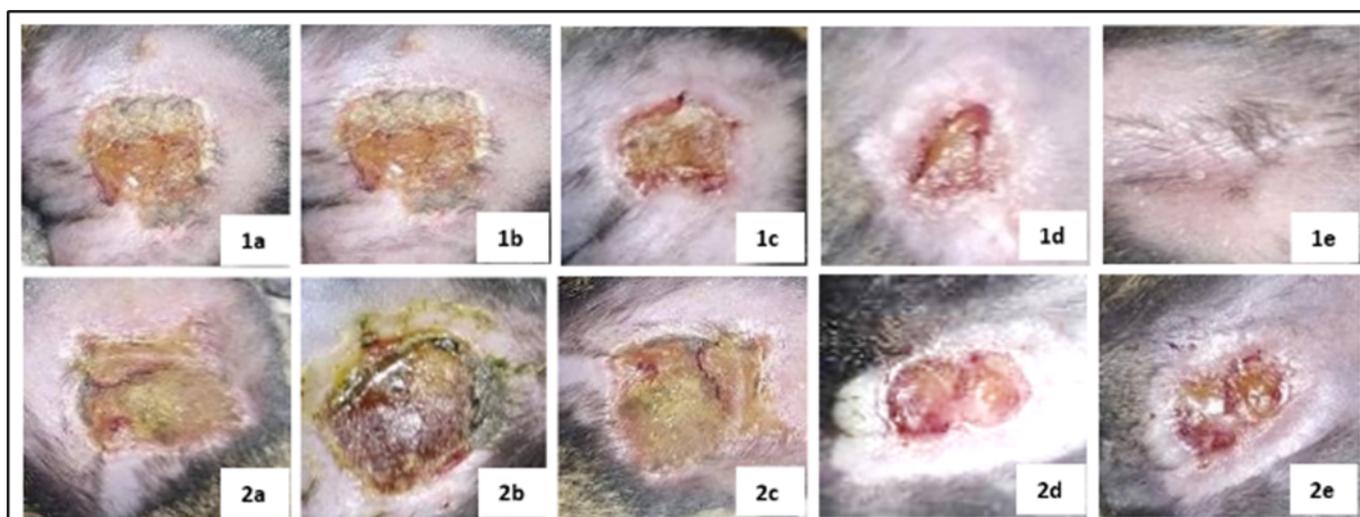


Figure 2. Second Degree Burns in Mice, 1= Bioplacenton Treatment as positive control (KP), a Day 3, b. Day 6, c. Day 9, d. Day 12, e. Day 15; 2 = Treatment 2, 1% *T. procumbens* and 0.5% *A. vera* leaf extract (P2), a. Day 3, b. Day 6, c. Day 9, d. Day 12, e. Day 15.

Discussion

The gel formulations in this study were evaluated for color, shape, and aroma (Table 4). The combined gel of *Tridax procumbens* and *Aloe vera* extracts appeared clear in treatments P1 (0.5% each) and P2 (1% *T. procumbens* and 0.5% *A. vera*). Increasing *T. procumbens* extract resulted in a more noticeable green hue due to its color. In contrast, the KP (Bioplacenton®) and KN (gel base) treatments remained clear. All gel preparations were semisolid and transparent. Both Bioplacenton® and the gel base had a distinct aroma, while the gel with *T. procumbens* and *A. vera* extracts had a stronger leaf scent than the controls. Evaluation of the homogeneity of the gel preparation was carried out using an objective glass. The homogeneity evaluation results showed that the positive control (Bioplacenton®) and negative control (gel base) preparations were homogeneous. In contrast, the gel containing *T. procumbens* and *A. vera* leaf extract looked inhomogeneous because it still had fine grains of the extract (Table 4).

The gel preparation was evaluated for its organoleptic properties and homogeneity before being

applied to the burn wound. Sulistyowati (2023) noted that gel formulations were chosen for their moisturizing properties, resulting in easy spreading and uniform distribution, preventing marks and thick layers. The cooling effect of the gel penetrates deeper than creams, and its blendability and absorption are superior to creams. In this study, the initial and final body weights of the mice showed no significant changes, indicating stability (Table 5), which may affect treatment outcomes. Nafi et al. (2020) suggest that body weight fluctuations can hinder wound healing. Despite the second-degree burns, the stable body weight is likely due to a diet that met their nutritional needs without compromising palatability. An optimal diet for mice should include 12% protein, 5% fat, and about 5% crude fiber, along with sufficient vitamins and essential amino acids (Mutiarahmi et al., 2021).

The histogram in Figure 1 shows that a gel combining *T. procumbens* and *A. vera* leaf extract (P1 and P2) affects the healing duration and process of second-degree burns in mice. The most effective treatment was Bioplacenton® (KP), achieving a 76.71% healing rate by day 12 and complete healing by day 15 (Figure 2). Bioplacenton® is used for burns due to its bactericidal properties, containing 0.5% Neomycin Sulfate and 10% placenta extract. Neomycin sulfate, derived from *Streptomyces fradiae*, is a broad-spectrum antibiotic effective against bacteria. It consists of two active isomers, B and C, and a minor component, neomycin A (Ivanalee et al., 2018). Neomycin Sulfate is indicated for burns, chronic ulcers, slow-healing wounds, granulations, impetigo, and other skin infections (Abdulkadir et al., 2023). Impetigo is a contagious but mild skin infection common in children, often resolving within days.

Bioplacenton® contains placenta extract that promotes cell regeneration and enhances collagen production, tissue protein levels, neoangiogenesis, and epithelialization. It is rich in enzymes like alkaline and pantothenic acid, biotin, PABA, folic acid, B12, choline, and inositol, along with amino acids, fatty acids, and essential elements such as Na, K, Ca, Mg, Cu, Fe, P, and Si. A study by Rinawati et al. (2021) found that Bioplacenton® was significantly more effective than kanyere leaf gel in healing burn wounds in rats, with a 43.21% reduction in inflammation and burn area compared to only 27.71% with kanyere leaf extract. This indicates that Bioplacenton® is more effective in reducing inflammation and accelerating burn wound healing, likely due to its placenta extract that promotes faster cell proliferation.

The P2 treatment group, containing 1% *T. procumbens* and 0.5% *A. Vera* leaf extract, showed the second-best results for burn wound healing, achieving a 65.62% healing rate by day 15 and complete closure by day 18 (Figure 2). In comparison, the P1 and KP groups healed by day 21 (Figure 1). Many plants, including *A. vera*, are known for their wound healing properties due to active compounds. *Aloe vera* gel contains saponins, flavonoids, tannins, and polyphenols (Samirana et al., 2020), while *T. procumbens* leaves are rich in various beneficial compounds (Debeturu et al., 2022).

Saponins in *A. vera* accelerate healing by acting as antibacterial agents, promoting new cell growth, and enhancing TGF- β activity, which aids in collagen synthesis and reduces pain (Alepandi et al., 2022; Kurniawan and Layal, 2017). Flavonoids have antimicrobial and antiviral properties, inhibit skin bleeding, and possess anti-inflammatory, anti-allergic, and anticancer effects, while also acting as antioxidants to reduce free radical damage (Wijaya et al., 2024; Kurniawan and Layal, 2017). They effectively combat

bacteria in wounds, aiding the healing process by damaging bacterial cell walls and inhibiting motility (Wardani, 2020). Additionally, flavonoids promote blood circulation, prevent vessel blockage, and alleviate pain and inflammation during bleeding or swelling (Safitri, 2022). They also benefit skin pigmentation by acting as tyrosinase inhibitors (Kurniawan and Layal, 2017).

Tannins, another type of polyphenolic compound, accelerate new tissue formation, protect against infections, and act as astringents to tighten skin and promote wound closure (Hakim et al., 2021; Priamsari and Yuniawati, 2019; Wijaya et al., 2024).

Wound healing is a complex process involving various cellular and biochemical activities aimed at restoring damaged tissue. It is influenced by several factors, including medication type, and consists of sequential stages: inflammation, cell proliferation, matrix deposition, and remodeling. Growth factors and cytokines play a crucial role in these phases (Rusdy et al., 2021). Burn wound healing occurs in three phases: inflammatory, proliferation, and maturation. The inflammatory phase lasts 3-4 days, characterized by vascular responses like edema and warmth. The proliferation phase focuses on repair, with fibroblasts migrating to the wound to synthesize collagen and other proteins essential for new tissue formation. This phase ends when the dermal epithelium and collagen layer are established, followed by the maturation phase, which strengthens the new tissue (Putri, 2022).

CONCLUSION

Mice with second-degree burns healed completely by day 18 using a gel with 1% *Tridax procumbens* and 0.5% *Aloe vera*, so this gel formulation presents a viable alternative for burn treatment. However, Bioplacenton® gel accelerated healing, achieving full recovery by day 15.

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