



EVALUATING THE EFFICACY OF TOPICAL MORINGA OLEIFERA AND ALOE VERA EXTRACTS IN ACCELERATING SECOND-DEGREE BURN WOUND HEALING

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DOI: <https://doi.org/10.33394/bioscientist.v12i2.13071>

Submit: 07-09-2024; Revised: 08-10-2024; Accepted: 12-10-2024; Published: 30-12-2024

ABSTRACT: Burn injuries are a global health issue, particularly second-degree burns, which damage both the epidermis and dermis. This has led to the exploration of natural treatments like *Moringa oleifera* (moringa) and *Aloe vera* for their potential in wound healing, pain relief, and infection prevention. This study aimed to evaluate the efficacy of a topical formulation containing extracts of *Moringa oleifera* and *Aloe vera* in accelerating the healing of second-degree burn wounds. This study employed a true experimental design with Sprague Dawley rats, divided into four groups (control positive, control negative, and two treatment groups). The experiment utilized topical formulations combining different concentrations of *Moringa oleifera* and *Aloe vera* extracts. The wound healing process was assessed through macroscopic observation and histopathological analysis, measuring the diameter of the burn wounds at different time intervals. Results showed that formulations containing *Moringa oleifera* and *Aloe vera* extracts significantly improved wound healing compared to the control group. The formulation with a 50:50 ratio of *Moringa oleifera* and *Aloe vera* demonstrated the highest efficacy, reducing wound size and promoting fibroblast proliferation and vascularization. This study confirms the potential of a topical combination of *Moringa oleifera* and *Aloe vera* extracts in accelerating the healing of second-degree burns.

Keywords: *moringa oleifera*, *aloe vera*, wound healing, natural extract, burn injury

How to Cite: Fauziah, D., Ramadani, N., Moniz, A., Syafitri, W., Humairah, S., & Pradipta, R. (2024). Evaluating the Efficacy of Topical *Moringa Oleifera* and *Aloe Vera* Extracts in Accelerating Second-Degree Burn Wound Healing. *Bioscientist: Jurnal Ilmiah Biologi*, 12(2), 1913-1925. <https://doi.org/10.33394/bioscientist.v12i2.13071>



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INTRODUCTION

Burn injuries are a significant global health issue, contributing to millions of cases each year, with severe implications for both individuals and healthcare systems (Khan et al., 2018). Of particular concern are second-degree burns, which affect both the epidermis and dermis, often resulting in prolonged healing times, an elevated risk of infections, and considerable pain and scarring. These complications not only affect the physical health of patients but also their psychological and social well-being (Alajmi et al., 2021). Moreover, patients recovering from second-degree burns often face challenges such as reduced mobility and disfigurement, impacting their quality of life.



Conventional treatments, including the widespread use of silver sulfadiazine, have demonstrated effectiveness in managing burn wounds. However, such treatments come with limitations, such as adverse effects like delayed wound healing and the growing concern of antimicrobial resistance, which poses a significant challenge in modern wound care (Avsar et al., 2016; Mastuti et al., 2023). This has led to an urgent need for alternative therapies that can not only expedite the healing process but also manage pain and prevent infections without contributing to antimicrobial resistance.

Natural-based therapies have garnered significant interest in recent years due to their potential in wound healing, pain management, and infection prevention (Georgescu et al., 2016). Among these, *Moringa oleifera* and *Aloe vera* have emerged as promising candidates for burn wound management. *Moringa oleifera*, also known as the "miracle tree," is rich in bioactive compounds such as flavonoids, phenolic acids, and vitamins, which have been shown to stimulate tissue regeneration and collagen synthesis, thereby enhancing the wound healing process (Jindal et al., 2021; Mawaddani et al., 2022). On the other hand, *Aloe vera* is well-known for its anti-inflammatory and antimicrobial properties, with polysaccharides and enzymes that promote wound debridement, angiogenesis, and re-epithelialization, all of which are critical for wound repair (Retnowati et al., 2021).

Moringa oleifera is also known to have antimicrobial properties, which help prevent infection in the wound area. Infection is one of the major complications in healing burns, and *Moringa oleifera* has the ability to inhibit the growth of pathogenic microorganisms, thereby contributing to a faster and cleaner healing process. A study by Al-Ghanayem et al. (2022) also found that *Moringa oleifera* leaf extract increased vascularization and fibroblast proliferation in rat wounds, strengthening its role in burn wound healing. (Al-Ghanayem et al., 2022).

Meanwhile, *Aloe vera* has long been used in traditional medicine to treat various types of wounds, including burns. Gel from *Aloe vera* leaves contains polysaccharides that promote epithelialization and angiogenesis, key processes in wound healing. Polysaccharides, along with vitamins and enzymes in *Aloe vera*, help debride wounds, clearing dead or damaged tissue, thereby accelerating the formation of new, healthy tissue. A study by Retnowati et al. (2021) showed that the use of *Aloe vera* gel in diabetic rats helped accelerate the wound healing process by increasing collagen synthesis (Retnowati et al., 2021).

Although the individual benefits of *Moringa oleifera* and *Aloe vera* in wound healing have been well-documented, limited research has explored the potential synergistic effects of combining these two extracts, particularly in the treatment of second-degree burns. Investigating this combination could provide valuable insights into alternative treatment methods that are safe, effective, and accessible, particularly in regions where conventional treatments may be expensive or unavailable. By evaluating the efficacy of a topical formulation containing both *Moringa oleifera* and *Aloe vera*, this study aims to contribute to the development of alternative therapies for burn wound management that reduce healing time, alleviate pain, and prevent infection, without the drawbacks associated with conventional treatments.

METHOD

This research design is a type of pure research (true experiment), with the research design of the randomized post-test only control group design. In this design, the experimental group is given treatment while the control group is not. All groups do not start with a pre-test. The sample in this study was white rats (*Rattus norvegicus*) that met the inclusion criteria. The sampling technique in this study used probability sampling with the simple random sampling method. Determination of the minimum number of samples in true experiment research uses the provisions by Federer (1999) with the formula $(n-1) (t-1) \geq 15$. The minimum number of samples needed is seven white rats (*Rattus norvegicus*) of the Sprague dawley strain in each group. In this study, there were four groups, so the total number of samples in this study was 28 which had been adjusted to the inclusion criteria set.

The inclusion criteria that researchers have determined are: (1) White rat (*Rattus norvegicus*) Sprague dawley strain; (2) The same age, namely ± 3 to 4 months; (3) Weight 150 - 200 grams; (4) The same sex, namely male; (5) In a healthy condition, it is characterized by active movement and not isolating itself in the corner of the cage, clean and smooth feathers, clear eyes, no abnormal discharge from the eyes, ears, anus and no defects and no weight loss of up to 10% during the acclimatization period; (6) Induced superficial second-degree burns. Whereas the exclusion criteria in this study are: (1) Disabled; (2) BB < 150 grams and > 200 grams; (3) Female gender; (4) White mice that died in the study.

The test was conducted in vivo with male rats of the *Rattus norvegicus* strain aged 2-3 months and weighing 150-250 grams. The number of samples was 40 rats divided into 5 groups. This research was conducted on rats that had been conditioned to have second-degree burns. Observation of wound healing activity was carried out macroscopically. Macroscopic observation by observing the diameter of the wound every 7 days and compared with the control group.

The next step is to determine the research subjects according to the predetermined sample requirements. Then the samples were grouped into 5 groups randomly. After the groups were formed, the experimental animals were adapted in the cage for 7 days. The cage was made of a plastic container measuring 55 cm x 35 cm x 15 cm with a rice husk base and the top was covered with strong, bite-resistant, non-damaged wire mesh so that the animals did not easily escape. In addition, the cage was placed in a room with good ventilation, sufficient light, quiet, not noisy, the temperature was set at room temperature around 25°C with a humidity of around 50%. The cage was cleaned every 3 days. Each cage contained 3-4 white mice. White mice were given a diet 3x / day with a protein composition of 4%, 5% fat and 3800 kcal / gram of crude fiber and mineral water for drinking given ad libitum.

The next stage is to make a shallow second-degree burn model on the gluteus medius muscle of a white rat. Anesthesia is needed on the experimental animal before performing the procedure of making a shallow second-degree burn to increase the comfort of the experimental animal. The procedure for anesthetizing experimental animals in Septafani (2011) begins by first determining the area to be burned, namely the back area of the white rat. The area where the wound is made

is shaved ± 4 cm². After that, disinfection is carried out on the skin area that will be burned using 70% alcohol cotton swabs. Then local anesthesia is carried out using 1% lidocaine as much as 0.1 ml subcutaneously in the area where the shallow second-degree burn model will be made.

Making a shallow second-degree burn wound model on a white rat using a smoothed metal nail head. The metal nail is heated in boiling water at a temperature of 80-100°C for ± 5 minutes. After the anesthetic works (± 30 seconds after administering local anesthesia), with the help of a corset, the hot metal is attached to the back of the white rat for ± 10 seconds until a blister forms on the skin. Making a shallow second-degree burn wound is done using sterile techniques to minimize secondary infection in burns. The process of making experimental animal models is carried out in the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. In the process of making histopathological preparations accompanied by a supervisor and laboratory assistant. Histopathological tests were carried out at the Animal Research and Diagnostic Laboratory of the Healthy Animal Clinic by observing histopathological preparations of testes in mice that were given treatment, namely the characteristics of damage scoring at 5LP (Field of View) different from 400x magnification which were then averaged, which were carried out direct observation of the image and automatically using Image Raster software. This observation uses a light microscope (Nikon Eclipse type Ei) with the help of an Outilab Microscope Camera connected to a computer.

The next process was preparing the treatment of experimental animals. After the blisters are formed, the wounds are compressed using normal saline. In this experiment, there are two control groups, namely positive control, and negative control. Both groups do not contain moringa leaf extract or aloe vera extract. The function of this control is as a comparison to assess the effects of the formulation being tested. Table 1 states that the experiment involved three variations of formulations labeled as F1, F2, and F3. Each formulation has a different dose combination of moringa leaf extract and aloe vera extract as follows:

1. **Formula 1 (F1)** consists of 1.0 grams of moringa leaf extract and 9.0 grams of aloe vera extract.
2. **Formulation 2 (F2)** contains 2.5 grams of moringa leaf extract and 7.5 grams of aloe vera extract.
3. **Formulation 3 (F3)** includes 5.0 grams of moringa leaf extract and 5.0 grams of aloe vera extract.

With this variation of formulation, the study aims to determine the optimal dose of the combination of moringa leaf extract and aloe vera that provides the best effect. The results of each formulation will be compared with positive and negative controls to assess the differences and effectiveness of each dose combination. Tween 80 functions as a surfactant to help form nanoemulsions. Polyethylene glycol, methyl paraben, and BHT are used as additional ingredients to increase the stability and durability of the formulation. Isopropyl myristate acts as a solvent, while phosphate buffer is used to maintain the pH of the formulation. The burns are then covered with gauze and taped with a non-irritating plaster so that the test animals do not scratch, remove, eat or lick the applied gel.



Table 1. Nanoemulsion Serum Formulation

Component	Positive control	Negative control	F1	F2	F3
Moringa Leaf Extract	-	-	1.0 grams	2.5 grams	5.0 grams
Aloe Vera Extract	-	-	9.0 grams	7.5 grams	5.0 grams
Tweens 80	-	-	30.0 mL	30.0 mL	30.0 mL
Polyethylene Glycol	-	40.0 mL	40.0 mL	40.0 mL	40.0 mL
Methyl Paraben	-	0.03 grams	0.03 grams	0.03 grams	0.03 grams
BHT	-	0.01 grams	0.1 grams	0.1 grams	0.1 grams
Isopropyl Myristate	-	10.0 mL	10.0 mL	10.0 mL	10.0 mL
Phosphate Buffer	-	20.0 mL	20.0 mL	20.0 mL	20.0 mL

Measurement of the effectiveness of burn healing includes the length of time required to form a new epithelial layer covering the superficial second-degree burn wound in each group of experimental animals observed every day. The length of time obtained is then calculated as an average for each group. When the superficial second-degree burn wound is completely covered by a new layer of skin without scar tissue formation, histopathological observation will be carried out. The experimental animals are euthanized via inhalation in a closed container containing cotton that has been soaked in excess chloroform for a few seconds. After that, the skin of the experimental animals is taken to make histopathological preparations.

RESULT AND DISCUSSION

In this section, the results of the study will be explained. The results of in vivo tests on white mice that have been conditioned with second-degree burns.

Table 2. In Vivo Test Results of Moringa Leaf Extract and Aloe Vera Leaf Extract on Mice with Second Degree Burns

Group	Wound Diameter (cm) day-			
	0	7	14	21
Positive Control		-	-	-
Negative Control	3.0	2.68	2.12	1.78
F1	3.0	2.70	2.52	2.44
F2	3.0	2.52	1.98	1.84
F3	3.0	2.14	1.84	0.72

The data in Table 2 presents the results of in vivo tests of administration of moringa leaf extract and aloe vera leaf extract to mice with second-degree burns. The data shown include wound diameter (in centimeters) measured on days 0, 7, 14, and 21 for various treatment groups, namely positive control, negative control, and three formulations (F1, F2, and F3).

In the positive control group, the wound diameter remained constant at 3.0 cm from day 0 to day 21, indicating no significant change or wound healing without any treatment. In the negative control group, the initial wound diameter was 3.0 cm

on day 0. After standard treatment without extract, the wound diameter decreased to 2.68 cm on day 7, 2.12 cm on day 14, and 1.78 cm on day 21, indicating a natural healing process. In group F1, which was given the first formulation, the wound diameter started from 3.0 cm on day 0, decreased to 2.70 cm on day 7, 2.52 cm on day 14, and 2.44 cm on day 21. This indicates slower wound healing compared to the negative control. In group F2, the initial wound diameter was 3.0 cm on day 0. Wound healing occurred faster than F1, with a reduction in wound diameter to 2.52 cm on day 7, 1.98 cm on day 14, and 1.84 cm on day 21. Group F3 showed the most significant results in wound healing. The wound diameter decreased from 3.0 cm on day 0 to 2.14 cm on day 7, 1.84 cm on day 14, and only 0.72 cm on day 21, indicating that this formulation is the most effective in accelerating the healing process of second-degree burns in mice.

Furthermore, in the histopathology test using skin organs to determine the wound healing effect based on indicators of increased epithelialization and increased collagen formation. Visually, the amount of vascularization and the number of fibroblast cells can be seen in Figure 1 and Figure 2.

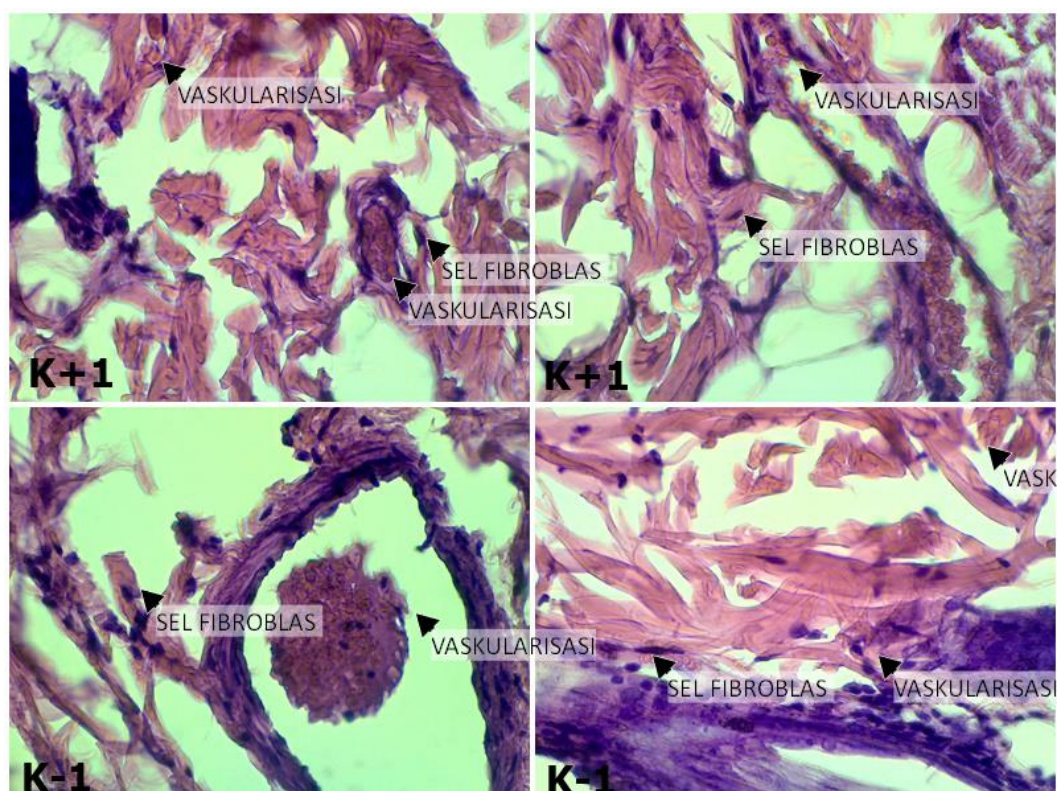


Figure 1. Morphology of Vascularization of Mouse Cells and Fibroblast Cells in the K- and K+ Groups.

Figure 1 presents the histopathological analysis of skin tissue in the wound healing process. The images show the morphology of vascularization and fibroblast cells in the K- (negative control) and K+ (positive control) groups. In the K+ group (top two images), there is a clear increase in vascularization, as indicated by the presence of well-formed blood vessels (marked as "Vaskularisasi") within the

tissue. The fibroblast cells (marked as "Sel Fibroblas") are densely packed, which is indicative of active tissue regeneration. The increase in both vascularization and fibroblast cells reflects enhanced wound healing, with increased blood supply supporting the delivery of nutrients and oxygen to the wound site, promoting tissue repair and collagen formation. In contrast, the K- group (bottom two images) shows reduced vascularization and fewer fibroblast cells. The blood vessels are less pronounced, and the arrangement of fibroblast cells appears less organized compared to the K+ group. This suggests slower wound healing, with limited angiogenesis and fibroblast activity, leading to delayed tissue regeneration and collagen synthesis.

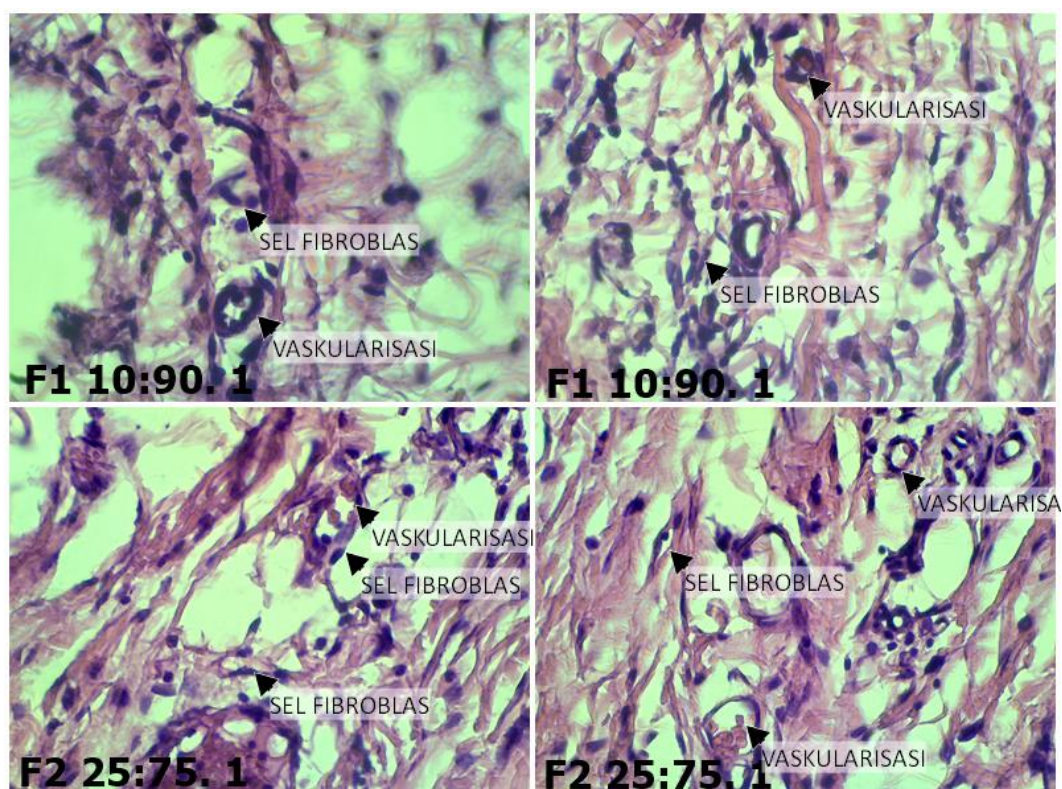
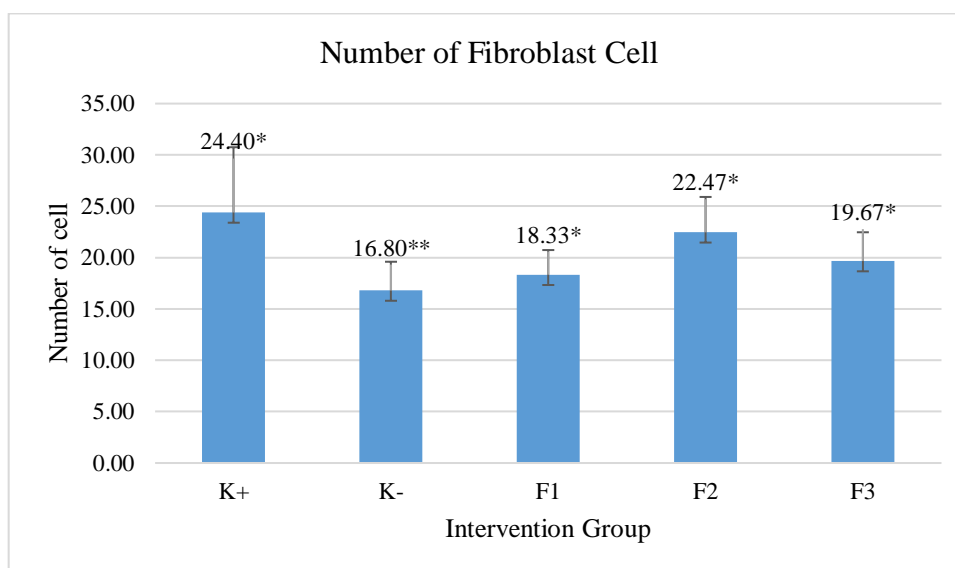


Figure 2. Morphology of Mouse Cell Vascularization and Fibroblast Cells in Groups F1 and F2.

Figure 2 illustrates the histopathological morphology of vascularization and fibroblast cells in the skin tissue of mouse models from the F1 (top two images) and F2 (bottom two images) groups. In the F1 group (top images), with the formulation of 10:90 (*Moringa oleifera* extract to *Aloe vera* extract), there is notable vascularization (labeled as "Vaskularisasi") and the presence of fibroblast cells (labeled as "Sel Fibroblas"). The tissue displays moderate vascularization, indicating the formation of blood vessels, which is essential for nutrient and oxygen delivery to the wound site, aiding in tissue repair. The fibroblast cells are visible, suggesting an ongoing process of collagen formation and wound healing, although it appears less dense compared to other formulations. In the F2 group (bottom images), with the formulation of 25:75 (*Moringa oleifera* to *Aloe vera*), the

vascularization appears more pronounced, with more well-formed blood vessels, indicating a higher level of angiogenesis compared to the F1 group. The fibroblast cells are also more abundant and densely packed, which suggests a stronger response in terms of collagen synthesis and tissue regeneration. This indicates that the 25:75 ratio may be more effective in enhancing the healing process, promoting both vascularization and fibroblast activity more efficiently than the 10:90 ratio.

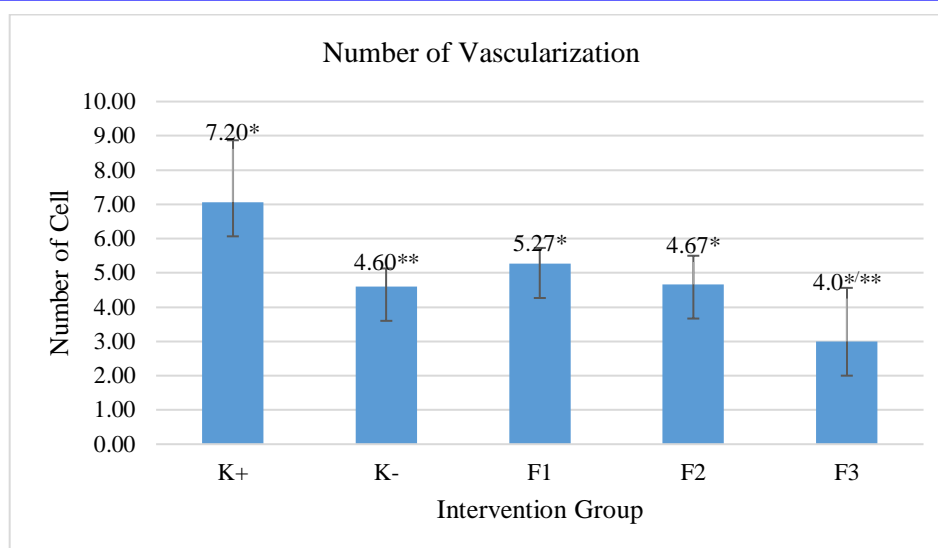


*significant to K- (p 0.05)

**significant to K+ (p 0.05)

Figure 3. Number of Fibroblast cells in the intervention group

The data in Figure 3 explains that positive control (K+) has the highest number of fibroblast cells (24.40). F1, F2, and F3 have statistically significant differences compared to K- ($p < 0.05$), indicating that the formulations containing moringa leaf and aloe vera extracts increase the number of fibroblast cells compared to the negative control. While K- has the lowest number of fibroblast cells (16.80). This is statistically significant compared to K+ ($p < 0.05$), which means the positive control also significantly increases the number of fibroblast cells. The formulations containing moringa leaf and aloe vera extracts (F1, F2, and F3) effectively increase the number of fibroblast cells compared to the negative control (K-). The positive control (K+) also shows a significant increase in fibroblast cells, suggesting that the positive control substance is effective.



*significant to K- (p 0.05)

**significant to K+ (p 0.05)

Figure 4. Number of Vascularization in the intervention group

The data in Figure 4 explains that positive control (K+) has the highest number of vascularization (7.20), which is significantly different from K- ($p < 0.05$). K- has the lowest number of vascularization (4.60), which is significantly different from K+ ($p < 0.05$). F1, F2, and F3 all show a significantly lower number of vascularization compared to K+ ($p < 0.05$), as indicated by the double asterisk (**) above the bars. F1, F2, and F3 all show a significantly higher number of vascularization compared to K- ($p < 0.05$), as indicated by the single asterisk (*) above the bars. Formulations 1, 2, and 3 (F1, F2, F3) effectively increase the number of vascularization compared to the negative control (K-).

The significant increase in fibroblast cells observed in the F1, F2, and F3 groups compared to the negative control (K-) indicates that these formulations successfully stimulate fibroblast proliferation. Fibroblasts play a critical role in the wound healing process by synthesizing collagen and contracting the wound, both of which are essential during the proliferative phase of healing (Cialdai et al., 2022). This finding aligns with previous research highlighting the wound healing properties of *Moringa oleifera* and *Aloe vera*, which are attributed to their antioxidant, anti-inflammatory, and tissue-regenerative effects (Al-Ghanayem et al., 2022; Mohammad Shafie et al., 2022b).

One study, conducted by Al-Ghanayem et al. (2022), explored the wound healing effects of *Moringa oleifera* in diabetic rats. This research demonstrated significant improvements in wound contraction, collagen formation, and increased capillary density, indicating the extract's strong antioxidant and proliferative properties. This may be similar to the results you're referencing regarding *Moringa oleifera*'s ability to promote fibroblast proliferation and enhance wound healing (Almehayawi et al., 2024). Another scoping review, published by Nurmaziah Mohammad Shafie et al. (2022), examined 18 in vivo studies that investigated *Moringa oleifera*'s wound healing properties. These studies consistently reported



positive outcomes in wound contraction and epithelialization, attributed to *Moringa oleifera*'s antimicrobial, antioxidant, and anti-inflammatory effects (Mohammad Shafie et al., 2022a).

The study on *Aloe vera* in promoting wound healing in diabetic rats does not appear to be easily accessible through the sources found. However, previous studies support the claim that *Aloe vera* gel can effectively promote wound healing by reducing wound size and increasing collagen synthesis (Hekmatpou et al., 2019). For example, *Aloe vera* has been shown to enhance re-epithelialization, which is critical in wound closure, and also promotes angiogenesis, contributing to improved nutrient and oxygen supply to the wound area. Studies such as those by and other reviews highlight that *Aloe vera* stimulates fibroblast proliferation, which supports tissue regeneration and accelerates the healing process, especially in diabetic and burn wounds. These findings align with your research's observations regarding the F3 group's enhanced vascularization (Haghani et al., 2022).

Although the F1, F2, and F3 formulations all increased vascularization compared to the negative control, they did not achieve the levels seen in the positive control (K+). Vascularization, or the formation of new blood vessels, is vital for providing oxygen and nutrients to the wound site, thereby supporting the healing process. This suggests that while *Moringa oleifera* and *Aloe vera* may contribute to angiogenesis, their efficacy may be lower than that of the positive control substance. Further investigation is needed to optimize the concentrations of these extracts to maximize their angiogenic potential.

Interestingly, while there were differences in fibroblast cell counts among the F1, F2, and F3 groups, vascularization remained relatively consistent across these groups. This suggests that the angiogenic response may not be directly dependent on fibroblast density within the range observed. Previous studies reinforce the notion that *Moringa oleifera* and *Aloe vera* possess valuable wound healing properties. This study builds on existing knowledge by examining the combined effects of these extracts in various formulations, providing insights into their potential synergistic interactions, and paving the way for the development of new wound healing therapies.

The beneficial effects observed in this study can be attributed to the anti-inflammatory, antioxidant, and antimicrobial properties of *Moringa oleifera* and *Aloe vera*. *Moringa oleifera* contains bioactive compounds such as flavonoids and phenolic acids that promote tissue regeneration by reducing oxidative stress and inflammation at the wound site. *Aloe vera*, on the other hand, contains polysaccharides that aid in debridement and epithelialization. These compounds likely worked in tandem to increase fibroblast proliferation and enhance vascularization, two key factors in effective wound healing. The increased vascularization observed in the F3 group is particularly important as it ensures a greater supply of oxygen and nutrients to the wound area, facilitating faster tissue repair.

Despite the promising findings, several limitations need to be acknowledged. The use of rat models limits the generalizability of the results to human patients, and further clinical trials are necessary to confirm these effects in human subjects. Additionally, while this study tested different concentrations of *Moringa*



oleifera and *Aloe vera*, the optimal formulation for human application remains to be determined. Moreover, the study focused solely on the short-term effects of these treatments, without examining long-term outcomes such as scar formation or recurrence of the wounds.

CONCLUSION

The study demonstrated that the combination of *Moringa oleifera* and *Aloe vera* extracts in varying formulations effectively enhanced the healing process of second-degree burns in experimental rat models. The formulation with equal parts of *Moringa oleifera* and *Aloe vera* (50:50) showed the most significant wound healing effect, indicated by reduced wound diameter, increased fibroblast cell proliferation, and enhanced vascularization compared to the control group. These findings suggest that natural extracts could serve as an alternative or complementary treatment for burn wounds, providing a safer and more affordable option compared to conventional treatments. Future studies should focus on optimizing the formulation, conducting clinical trials, and exploring the underlying mechanisms of the synergistic effects of these plant extracts in wound healing.

RECOMMENDATION

Further research is necessary to validate these findings in clinical trials involving human patients with burn wounds. Such studies could explore the optimal concentration and combination of *Moringa oleifera* and *Aloe vera* extracts to achieve the best wound-healing results. Additionally, future research should delve deeper into the molecular mechanisms underlying the observed effects, particularly focusing on how these natural extracts influence collagen synthesis, angiogenesis, and fibroblast proliferation at a cellular level.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Ministry of Research, Technology and Higher Education Indonesia for funding this research through the Student Creativity Program scheme.

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