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The Effect of Torch Ginger Flower Extract Cream (*Etlingera elatior*) on the Macroscopic and Histopathological Features of Wound Healing on Mice Skin

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Abstract | Background: Wound healing is a complex process of the body to repair damaged tissue. Torch ginger (*Etlingera elatior*) contains flavonoid quercetin, which is an antioxidant, antibacterial and anti-inflammatory. Aims: This study aimed to determine the effect of administering torch ginger flower extract cream (TGFEC) on the excision wound on the skin seen from the percentage of wound healing area, the average number of inflammatory cells, the percentage of collagen density area and the percentage of interleukin-6 immunoreactive area. Methods: The study used 24 female mice which were randomly divided into 4 treatment groups, namely K1 (negative control), K2 (TGFEC 2,5%), K3 (TGFEC 5%) and K4 (TGFEC 10%). Therapy using TGFE cream was carried out for 9 days, then euthanasiain was carried out using the cervical dislocation method and wound skin samples were taken on days 3, 6 and 9. Results: The average percentage of wound healing increased in wound closure after treatment in group K1, K2, K3 and K4. The number of inflammatory cells in the treatment group was lower than the control group on day 3 and day 6, but higher on day 9 compared to group K1. The average percentage of collagen density in the K2 and K3 were higher than K1 and K4 on days 3 and 6, but lower on day 9 compared to K1. K4 had the lowest average percentage of collagen density. The percentage of interleukin-6 immunoreactive area on day 9 showed that K2 had a low percentage of immunoreactive area, followed by K1, K3 and K4. On day 9 of treatment there was a significant difference ($p<0.05$) between K1 and K3; and K2 with K3 and K4. Conclusion: To conclude, the 2,5% torch ginger flower extract cream (K2) may offer potential as an alternative therapy for wound healing as seen from its ability to enhance the percentage of wound healing ($p>0.05$), reduce the average number of inflammatory cells ($p>0.05$), increase collagen density ($p>0.05$) and reduce interleukin-6 expression ($p<0.05$).

Keywords | Wound healing, Skin, Torch ginger flower, Histopathology, Interleukin-6, Mice, Herbal medicine, Antioxidants

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INTRODUCTION

The skin is the first line of defense in the body (Tottoli *et al*., 2020). As the first line of defence, the skin is often exposed to various external factors, making it very vulnerable to injury (Szalay and Wertz, 2023). Complex intracellular and intercellular mechanisms are stimulated after trauma or damage in order to immediately restore tissue homeostasis (Zomer and Trentin, 2018). Wounds result in loss of epithelial continuity with or without loss of basement membrane and dermis (Widyawati *et al*., 2019). Wound healing is a dynamic and complex process that occurs in stages: inflammation, re-epithelialization, granulation tissue formation, neovascularization, wound contraction, and extracellular matrix remodelling (Hakim *et al*., 2019). This wound healing serves to protect the body by restoring the integrity of damaged tissue. Rapid wound healing is essential to prevent infection and reduce complications and treatment costs (Rodrigues *et al*., 2019).

The wound healing process is regulated by various cells, cytokines and growth factors (Zomer and Tretin, 2018). One of the important cytokines in the wound healing process is Interleukin-6 (IL-6) (Li *et al*., 2022). Interleukin-6 is a pleiotropic cytokine involved in the growth and differentiation of various cell types in the wound healing process (Seaton *et al*., 2015). Johnson *et al*. (2020) state that IL-6 plays an important role in acute inflammation and the duration of wound healing. IL-6 expression in normal wound healing will decrease in the remodelling phase (Li *et al*., 2022). This is associated with apoptosis of inflammatory leukocyte cells and reduction of cytokine signals (Tanaka *et al*., 2014). This low concentration of IL-6 helps accelerate wound healing; therefore, the concentration of IL-6 needs to be considered.

The torch ginger plant is a spice plant that has been used for generations as a herbal medicine and food flavouring. The torch ginger plant contains compounds of the phenol group, glycosides, flavonoids quercetin, apigenin, kaempferol, luteolin and myricetin found in torch ginger (Levita *et al*., 2019). Flavonoids are able to treat wounds and act as astringents and antimicrobials which can be responsible for wound contraction and increase epithelialization. Flavonoid content can help accelerate collagen growth (synthesize collagen) through increased fibroblasts and tissue formation (Kastika and Rahayu, 2018). Carvalho *et al*. (2021) explained that flavonoids have effects on the inflammatory process, angiogenesis, re-epithelialization and oxidative stress. Flavonoids can influence macrophages, fibroblasts and endothelial cells by mediating the expression of inflammatory mediators. Research by Chang *et al*. (2012), states that torch ginger flowers contain kaempferol-O-3-glucoside, quercetin and kaempferol. Kaempferol from torch ginger flowers has been shown to have anti-inflammatory activity and can protect against brain tissue damage, so it can be used for ischemic stroke therapy.

Previous studies have used various parts of the torch ginger plant as a wound healing medicine, such as leaf, fruit and flowers. Chang *et al*. (2020) used ethanol extract of torch ginger flowers as a topical therapy for burn model. In a study by Ginting (2022) used ethanol extract of torch ginger fruit. Efendi *et al*. (2020), used torch ginger leaf extract for incision wound. This study aimed to determine the effect of administering torch ginger flower extract cream on macroscopic and histopathological parameters of excision wound healing on mouse skin. The difference between this study and previous studies is that the wound model and wound healing observations were not only carried out macroscopically, but also microscopically examination.

MATERIALS AND METHODS

Ethical Clearance Approval

The Research Ethics Commissions of the Faculty of Veterinary Medicine, University of Gadjah Mada, Indonesia, approved this research under document number 101/EC-FKH/int./2024.

Animal Preparation

The research used 24 female Balb/c mice, aged 8 weeks with an average body weight of 20-30 grams. The mice were divided into four treatment groups with three different time points, with two mice each time point. Each mouse had two excisional wounds, resulting in four wounds in each group and 48 wounds in total. The mice were purchased from the Integrated Laboratory for Research and Testing at Universitas Gadjah Mada, Indonesia. The mice had a one-week acclimation period and kept individually in 12 hours light-dark cycle. They were given food and water *ad libitum* during the experiment.

Phytochemical Test

Phytochemical tests are used to identify and evaluate the presence of bioactive compounds in plants. This test is to explain how the plant mechanism works in wound healing and to determine the types of components that can trigger wound healing. Qualitative screening of the phytochemicals and quantitative determinations of the chemical constituents in the plants referred to journal Ajuru *et al*. (2017).

Experimental Design

The hair on the back of the mice were shaved one day before treatment began. Before the excision wound was made, the experimental animals were first anesthetized with an intraperitoneal injection of ketamine-xylazine with the anaesthetic dose 90 mg/kg BW ketamine and 10 mg/kg

BW xylazine. Furthermore, each mouse was made 2 excision wounds with a punch biopsy diameter of 6 mm and a distance between wounds of 1.5 cm (Yampolsky *et al*., 2024). Therapy using torch ginger flower extract cream was carried out for 9 days in the therapy groups. The cream was applied to the excision wound twice a day, with 0.1 ml applied each time. Macroscopic observation, by using measurement of the wound diameter and sampling of the wound skin was carried out on day 3, 6 and 9. Subsequently, mice were anesthetized with injection of ketamine-xylazine and then euthanized via cervical dislocation. According to AVMA (2020), cervical dislocation is acceptable for euthanasia mice weighing under 200 g. The skin samples were cut out and then fixed in 10% buffer formalin for histopathological examination as previously reported (Slaoui and Fietter, 2011).

Haematoxylin and Eosin Staining

Haematoxylin eosin staining was carried out by deparaffinizing the slides and rehydrating the slides by immersing the tissue samples in xylene I for 5 minutes, xylene II for 5 minutes and xylene III for 2 minutes. The next stage, the tissue samples were soaked successively in absolute alcohol I, absolute alcohol II, 95% alcohol, 95% alcohol, 70% alcohol for 1 minute each. The samples were soaked in haematoxylin solution for 10 minutes and dipped in water 4 times. After that, the sample was dipped in 1% alcohol acid 3-10 times, then rinsed with running water for 15 minutes. The next stage was that the sample soaked in eosin for 15 seconds to 2 minutes. Then the dehydration process was carried out, cleared and mounted. Haematoxylin Eosin staining was done according to the standard Pathology Laboratory procedures, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Masson's Trichrome Staining

The slides were deparaffinized and dehydrated using xylene and graded alcohol and then rinsed with tap water. Then added haematoxylin to the slide for 5-10 minutes to stain the cell nuclei and rinsed with water. The next stage was administering solution acid fuchsin onto the slide for 5-10 minutes with the aim of staining the cytoplasm and muscle red. Then rinsed with water and added the solution phosphomolybdic acid onto the slide for 10-15 minutes to release the color from the connective tissue, but did not release the color on the muscle and rinsed with water. Then added aniline blue or methylene blue for 5-10 minutes to stain the connective tissue blue. The last step was given acetic acid for 2 minutes. Finally, the slides were rinsed with tap water for 5 min and were subsequently dehydrated, cleared, and mounted (Digambiro and Purwanto, 2024). Staining was carried out according to standard procedures of Pathology Anatomy Laboratory, Faculty of Public Health Medicine and Nursing, Universitas Gadjah Mada.

Immunohistochemical Staning

Before incubation with the primary antibody, the slides were incubated using a retrieval solution (Bond Epitope Retrieval Solution, catalog number RE7119, Leica Biosystems) at 98°C for 20 min and rinsed with cold water. The slides were then incubated with 4% hydrogen peroxide (Peroxidase Block, catalog number RE7101, Leica Biosystems) for 5 min, rinsed with phosphate buffer saline (PBS), incubated with 0.4% casein in PBS (Protein Block, catalog number RE7102, Leica Biosystems) for 5 min and rinsed with PBS. The slides were then incubated with primary antibody IL-6 (Catalog number sc-130326; Santa Cruz Biotechnology Inc.) at 1:50 dilution for 30 min. Rabbit anti-mouse IgG in 10% animal serum (PostPrimary Antibody, catalog number RE7111, Leica Biosystems) was then applied on the slides for 30 min, rinsed with PBS, and then incubated with anti-rabbit poly-HRP IgG containing 10% animal serum (Novolink Polymer, RE7112, Leica Biosystems) for 30 min. Subsequently, the slides were then incubated with diaminobenzidine chromogen for 5 min, rinsed with tap water, and then counterstained with hematoxylin (Catalog number: RE7107, Leica Biosystems) for 30 s (Prakoso *et al*., 2020; Widyarini *et al*., 2023).

Macroscopic Study

Macroscopic data of the wound was obtained by measuring the diameter of the wound area using a calliper. Measurement of the diameter of the wound area was carried out every day from day 0 when the wound was made until day 9. The wound area was measured using the formula:

$$A=\pi \times x \times y$$

Annotation; A: Wound area (mm^2); X: x-axis of the wound (mm); Y: y-axis of the wound (mm).

The percentage of wound healing was measured using the formula:

$$\%WH = \frac{(a-b)}{a} \times 100\%$$

Annotation: %WH: Percentage of wound healing (%); a: initial wound area (mm^2); b: final wound area (mm^2).

Microscopic Study

Microscopic data of the wound was obtained by calculating the number of inflammatory cells in Haematoxylin Eosin staining skin tissue, collagen density in Masson Trichrome staining and interleukin-6 expression in immunohistochemistry staining. Inflammatory cells and collagen density were counted in three different fields of view that were randomly determined in the wound area. Observations were made using a light microscope with a magnifica-

tion of 400X (Olympus CX-43, Japan). The results of the calculation of the number of inflammatory cells were processed using Image Raster 3.0 software, while the calculation of collagen density and interleukin-6 expression were processed using ImageJ software (Paramanandi *et al.*, 2024).

Statistical Analysis

The data obtained were analysed statistically using SPSS 25 software with α: 0,05 The data was first tested for Shapiro-Wilk normality, the results of which were normally distributed (Sig.>0.05). Then continued with Levene's homogeneity test, the results of which were non-homogeneous data (Sig.>0.05). Data that was normally distributed and non-homogeneous were continued with non-parametric tests Kruskall Wallis and was followed by the Mann Whitney test.

RESULTS

Phytochemical Test

Torch ginger flower extract's compounds proven by the phytochemicals test such as quercetin 42,20 mg/kg; tannin 12,52 mg/kg; saponin 7,05 mg/kg; phenol 5,45 mg/kg and alkaloid 5,25 mg/kg.

Gross Pathology

The wound area was measured using a calliper at three time points included on days 3, 6, and 9 after treatment. The average percentage of wound healing showed an increase in wound closure after treatment in Groups K1, K2, K3 and K4. Moreover, after nine days of topical medication, groups K1, K2, and K3 showed the most wound closure (Figure 1). Topical treatment with torch ginger extract 2,5% cream (Group K2) showed smaller wound closure compared to the other treatment groups. However, statistical analysis demonstrated no significant difference between the treatment group ($p > 0.05$) on day 3, 6 and 9 (Table 1).

Table 1: The percentage of wound healing after treatment

Groups	Day-0 (%)	Day-3 (%)	Day-6 (%)	Day-9 (%)
K1	0	52,86	68,42	90,63
K2	0	38,64	66,38	90,53
K3	0	24,03	64,56	90,00
K4	0	26,85	64,93	79,55

Histopathology: Inflammatory Cells

Inflammatory cells were counted in three distinct areas of the dermis layer (Figure 2) for all groups. Overall, the average number of inflammatory cells after 3 and 6 days of therapy in the treatment groups declined, as compared to Group K1 (Table 2), indicating reducing inflammatory response in wound area. On day 9, the average number of inflammatory cells in the treatment group are higher than the control group (Group K1). Statistical analysis was performed using a non-parametric Kruskall-Wallis test and Mann Whitney test. However, statistical analysis revealed no significant difference in wound area between the groups ($p>0.05$) on day 3, 6 and 9 (Table 2).

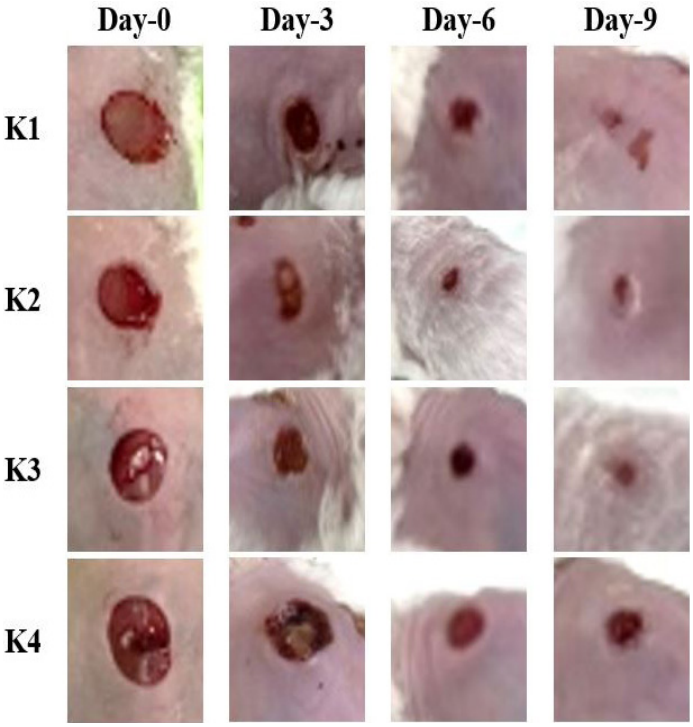

Figure 1: Wound healing after treatment with torch ginger extract cream. K1: without treatment; K2: cream with torch ginger extract 2,5%; K3: cream with torch ginger extract 5%; K4: cream with torch ginger extract 10%. On the first day (day 0), the wound appears reddish, moist and bleeding. After 3 days of treatment, the wound starts to form scab, swell surrounded the wound and no more bleeding. After 6 days of treatment, the wound area reduces on K1 and K2 group compared to K3 and K4 group. After 9 days of treatment, the wound area in K1 and K2 is closed but there is still redness. Conversely, the wound area of group K3 and K4 is wider than the K1 and K2 group.

Table 2: The average number of inflammatory cells at day 3, 6 and 9 after treatment with torch ginger extract cream

Groups	Day-3 (cell/three fields of observation)	Day-6 (cell/three fields of observation)	Day-9 (cell/three fields of observation)
K1	156,5±72,67	141±36,49	58,83±27,47
K2	106,83±18,85	124,58±16,27	75±22,12
K3	108,75±21,25	128,08±21,98	86,58±24,63
K4	89,33±25,26	118,58±15,68	116,67±40,23

Notes: The meaning of unit (cell/three fields of observation) is the average number of inflammatory cells in three microscope fields of observation. Each data was taken from three fields of observation at 400x magnification.

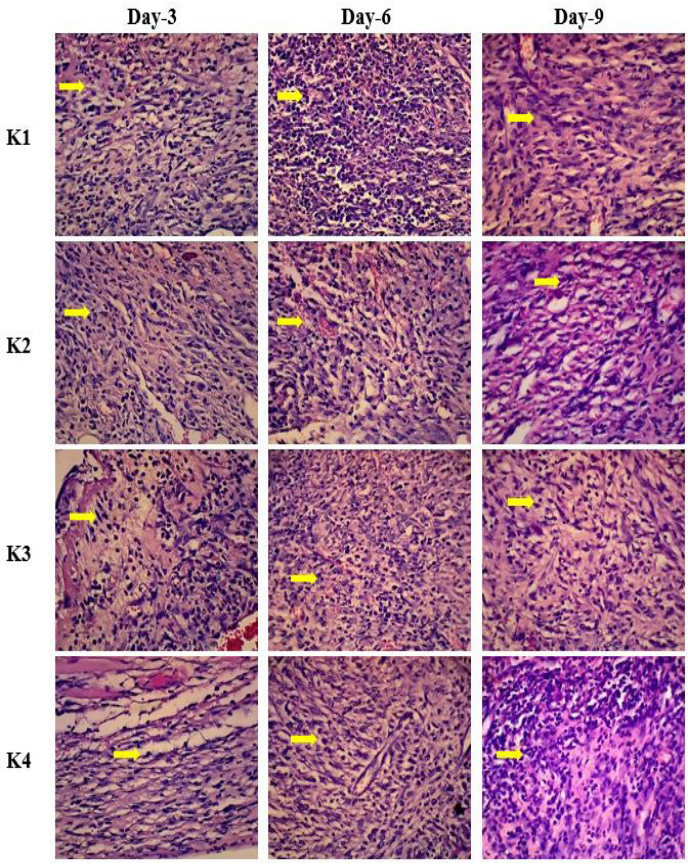

Figure 2: Microphotography of inflammatory cells in wound healing after treatment with torch ginger extract cream on days 3, 6 and 9. K1: without treatment; K2: cream with torch ginger extract 2,5%; K3: cream with torch ginger extract 5%; K4: cream with torch ginger extract 10% (Haematoxylin Eosin staining, 400x magnification). Inflammatory cells are visible as a dark coloured, wound, oval or elongated cells on the histopathological features which indicates the presence of inflammatory cells in wound area. Inflammatory cells mostly found in connective tissue in day 3 and decrease on each time points.

Table 3: The percentage of collagen density on days 3, 6 and 9 after treatment with torch ginger extract cream.

Groups	Day-3 (%)	Day-6 (%)	**Day-9 (%)**
K1	14,37 ±1,2	24,3±1,07	26,05±2,3*
K2	20,19±2,5	26,12±2,4	23,04±0,49
K3	18,82±3,9	26,47±0,38*	20,71±0,95*
K4	14,27±2,8	23,63±1,6*	20,72±1,8*

*): statistical significance (p<0,05).

Histopathology: Collagen Density

The density of collagen was assessed from three different regions using Trichrome Masson's staining (Figure 3). The percentage of collagen density in treatment groups K2 and K3 showed an increasing trend compared to Group K1 on day 3 and 6. Statistical analysis was performed using a non-parametric Kruskall-Wallis test and Mann Whitney test. There was no significant difference in the collagen density between KI and K2 (p>0.05). There was significant difference between Group K3 and K4 on day 6 and between Group K1 and K3 and K4 (p<0.05) on day 9 (Table 3).

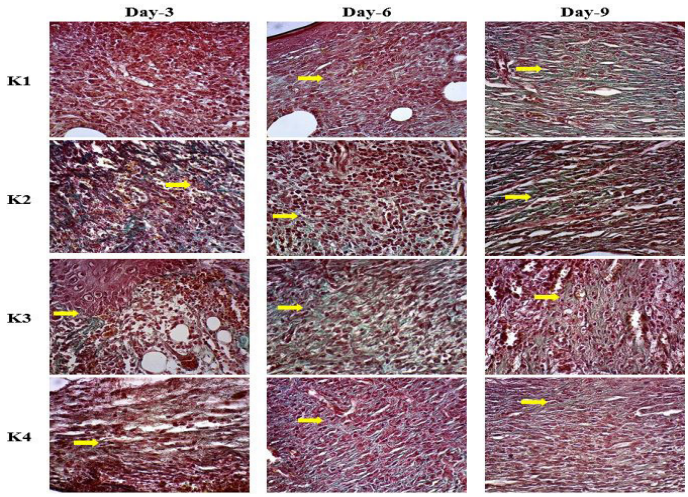

Figure 3: Microphotography of collagen density in wound healing after treatment with torch ginger extract cream on days 3, 6 and 9. K1: without treatment; K2: cream with torch ginger extract 2,5%; K3: cream with torch ginger extract 5%; K4: cream with torch ginger extract 10% (Masson Trichrome staining, 400x magnification). Collagen density is visible as a bluish area on the histopathology image which indicates the presence of collagen in that area. After 3 days of treatment, collagen has not formed yet because inflammatory cells still dominate in the connective tissue, as evidenced by the accumulation of inflammatory cells in the haematoxylin eosin staining. Collagen density appears denser in the treatment group using torch ginger extract cream 2,5% rather than the other groups after 9 days of treatment.

Table 4: The percentage of interleukin-6 expression on days 3, 6 and 9 after treatment with torch ginger extract cream

Kelompok	Hari ke-3 (%)	Hari ke-6 (%)	Hari ke-9 (%)
K1	18,01±9,9	28,40±10,6	30,38±11,8*
K2	26,83±3,3	40,51±11,9	19,46±3,8*
K3	23,37±6,8	27,71±14,5	40,36±2,7*
K4	27,88±9,6	33,55±8,7	45,07±3,5*

Notes: Interleukin-6 expression was measured by setting the threshold using ImageJ software. *): statistical significance (p<0,05).

Histopathology: Interleukin-6 Expression

The interleukin-6 expression was assessed from three different regions (Figure 4). Immunohistochemical analysis on day 3 showed that the average area of IL-6 immunoreactive in K2, K3 and K4 was higher than K1. On day 6 of treatment, the average area of IL-6 immunoreactive was highest in K2, followed by K4, K1 and K3. Statistical analysis was performed using a non-parametric Kruskall-Wallis

test and Mann Whitney test. Based on statistical analysis, there was no significant difference (p>0.05) between the four groups on day 3 and day 6. On day 9 of treatment, there was a significant difference (p<0.05) between K1 and K4; and K2 with K3 and K4. There was no significant difference in the expression of IL-6 between KI and K2 (P>0.05). Hence, the lowest concentration of torch ginger flower extract cream 2.5% resulted in expression of IL-6 similar to K1. The results of calculating the average percentage of interleukin-6 immunoreactive area can be seen in Table 4.

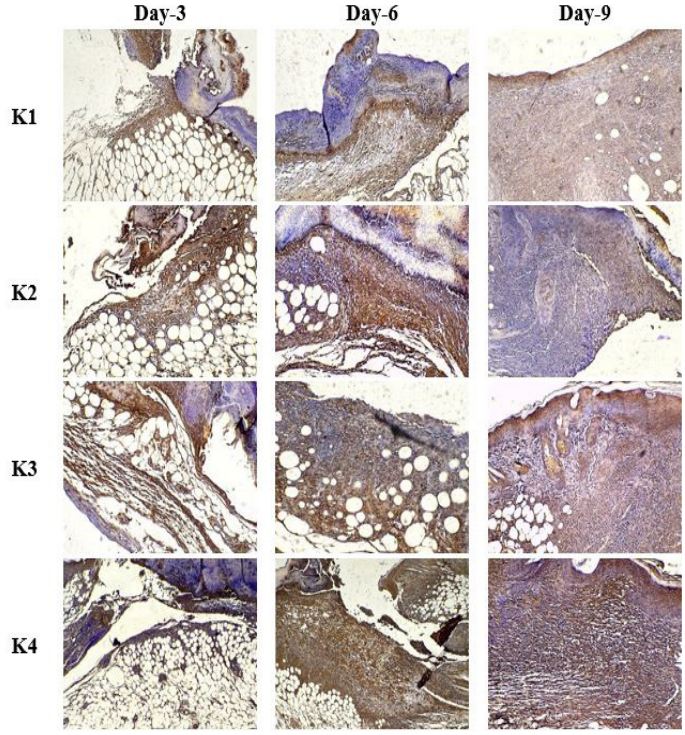

Figure 4: Interleukin-6 expression in wound healing after treatment with torch ginger extract cream on days 3, 6 and 9. K1: without treatment; K2: cream with torch ginger extract 2,5%; K3: cream with torch ginger extract 5%; K4: cream with torch ginger extract 10% (Antibody anti-IL-6 staining, 100x magnification). Interleukin expression is visible as a brownish area on the histopathology image which indicates the presence of interleukin 6 in that area. After 9 days of treatment, K2 has lower interleukin-6 expression compared to other groups.

DISCUSSION

This study aims to see the effect of administering 2.5%, 5%, and 10% torch ginger flower extract cream on excision wounds on the back skin of mice. The wound healing parameters observed were the percentage of wound closure, the number of inflammatory cells, collagen density and interleukin-6 expression.

The results of this study show that torch ginger flower extract cream is able to heal wounds by suppressing the inflammatory response, triggering collagen proliferation and increasing or decreasing the regulation of interleukin-6 expression. Torch ginger flower extract cream with a concentration of 2.5% is the best concentration in the wound healing process and causes a decrease in the regulation of interleukin-6. Downregulation of IL-6 indicates that the inflammatory phase has reduced so that the proliferation phase can begin.

The treatment group given torch ginger flower extract with various concentrations inhibits wound closure on day 3 compared to KI, however on day 6 and 9 the percentage of wound closure was not different from K1 (p>0.05). The lower percentage of wound closure of K2, K3, and K4 compared to K1 was in line with the results of histopathological examination, which showed that the average number of inflammatory cells in K2, K3, and K4 was lower than K1.

In this study, the lower number of inflammatory cells in K2, K3, and K4 on day 3 and day 6 might be related to anti-inflammatory activity of torch ginger flower as previously reported (Habsah *et al.*, 2005; Chang *et al.*, 2012). On day 9 of treatment there was no significant difference on the average number of inflammatory cells among treatment group.

First, compared with the negative control, K2 and K3 groups were able to suppress the inflammatory response. However, in the K4 group, there was a delayed inflammatory response. It is suspected that at high concentrations, the components in the extract are antagonistic. The mechanism at high concentrations is not clear. This requires further research to discuss in more depth. The increase in concentration is not directly proportional to the inflammatory response (the number of inflammatory cells) on days 3, 6 and 9. Second, skin samples were taken from different mice each day, so the inflammatory response that occurred also depended on the individual's immune response. However, when viewed from the number of inflammatory cells each day, K2 showed a lower number of inflammatory cells than the other groups.

The results suggest that the phytochemical compounds contained in torch ginger flower extract cream have an anti-inflammatory activity. The phenol, flavonoid and terpenoid components contained in TGEC have been proven to be able to inhibit the expression of pro-inflammatory cytokines such as *Tumor Necrosis Factor*-α (TNF-α), interleukin 1β and interleukin-6, as well as reducing the synthesis of prostaglandins and leukotrienes which are inflammatory mediators (Fawzy and Putranti, 2023). According to Ningsih *et al.* (2023), quercetin as an anti-inflammatory works by inhibiting the activity of the COX and lipoxygenase enzymes directly, thereby inhibiting the biosynthesis of prostaglandins and leukotrienes which are the final products of

the COX and lipoxygenase pathways. Inhibition of leukocytes during the inflammatory process causes a decrease in the body's response to inflammation (Fawzy and Putranti, 2023). The torch ginger flower extract cream is able to control the number of inflammatory cells in the early stages of inflammation without disrupting the duration of wound closure as seen from the percentage of wound closure on K1, K2, and K3 on day 9 which is not much different.

The transition from the inflammatory phase to the proliferative phase demonstrated by fibroblast invasion and increased collagen accumulation in the wound area (Williams and Moores, 2017). The proliferation phase occurs when new granulation and epithelial tissue is formed to restore blood circulation which functions to maintain the new tissue (Bodas and Shinde, 2021). Collagen is the main component of the extracellular matrix and is responsible for the strength of new tissue. There was no significant difference in the average percentage of collagen density on day 3 from all treatment group. There was no significant difference ($p>0.05$) between K1, K2, and K3 in the average of collagen density. Interestingly on day 9, the average of collagen density from KI and K2 was no significant difference. The average percentage of collagen density of K1 and K2 in line with the results of the average number of inflammatory cells, expression of IL-6, and wound closure. According to Velnar *et al*. (2009) and Paramanandi *et al*. (2024), fibroblast proliferation and collagen production will occur when the number of inflammatory cells has decreased. If leukocytes are hampered in the wound cleansing process and cannot be removed through apoptosis, the proliferation phase is delayed and the wound takes longer to heal (Holzer-Geissler et al., 2022). Contraction of the wound causes the edges of the wound to come together to ensure that the wound closes (Nirenjen *et al*., 2023). This process is an indication that the formation of granulation tissue, angiogenesis, infiltration of fibroblasts and keratinocytes in the wound was successful (Mattew-Steiner *et al*., 2021).

Interleukin-6 is an inflammatory modulator and plays a role in the process of differentiation, activation and proliferation of leukocytes, endothelial cells, keratinocytes and fibroblasts (Nosenko *et al*., 2019). Interleukin-6 is involved in phagocytosis, chemokine secretion, initiation of inflammatory responses and influences the duration of wound healing (Nirenjen *et al*., 2023; Widyarini *et al*., 2023). The results of calculating the interleukin-6 immunoreactive area in this study showed that there was no significant difference ($p>0.05$) between the four groups on day 3 and day 6. The percentage of immunoreactive area on day 9 showed that K2 had a low percentage of immunoreactive area, namely 19.46 ± 3.8 followed by K1 with a value of 30.38 ± 11.8, K3 40.36 ± 2.7 and K4 45.07 ± 3.5. There was no significant difference ($p>0.05$) between K1 and K2. The immunoreactive percentage area of groups K1, K3, and K4 on day 9 showed an increase in interleukin-6 expression compared to day 6. The increase in the immunoreactive area was possibly caused by the needs for interleukin-6 that released by inflammatory cells for inducing proliferation process in the wound area. Nirenjen *et al*. (2023) reported that an increase in IL-6 is related to the ongoing inflammatory process and will cause delayed wound healing. On day 9, the K2 group experienced a decrease in interleukin-6 immunoreactive area compared to the other groups. According to Johnson *et al*. (2020), IL-6 expression will decrease in phase remodelling in the normal wound healing process. Furthermore, the decrease in interleukin-6 expression is thought to be associated with apoptosis of leukocyte inflammatory cells and reduction of cytokine signals as previously reported (Tanaka *et al*., 2014). During the proliferation phase, IL-6 plays a role in the secretion of Keratinocyte Growth Factor (KGF) which functions for the growth and migration of keratinocytes on the wound surface. Interleukin-6 also increases the regulation of TGF-β in fibroblasts which results in increased collagen production (Zomer and Tretin, 2018).

Agents that can stimulate reepithelialisation, migration and proliferation of endothelial cells and fibroblasts, as well as increasing collagen synthesis are considered wound healing agents (Boadi *et al*., 2024). The torch ginger flower extract cream 5% and 10% caused an inhibition of the percentage of wound closure, the average number of inflammatory cells was still high on day 9, the percentage of collagen density was low and the percentage of interleukin-6 expression was quite high. It might be due to dermal toxicity in mouse skin as previously reported (Riana *et al*., 2023). Dermal toxicity refers to the toxic effects that can occur on the skin due to the use of topical medications. The response to extract concentration increases in direct proportion to increasing concentration (Wexler, 2024). If the treatment response is maximal and the metabolites compound attached at a concentration that is too high, then increasing the concentration no longer causes a therapeutic response. This is thought to cause the wound healing effect of 5% and 10% torch ginger flower extract cream to not be as good as cream with a concentration of 2.5%.

Medicines derived from plants are known to be safe and are thought to have minimal side effects. This statement is not always correct because chemical compounds at high doses can act as toxins (Pola and Rada, 2023). Plants contain a variety of secondary metabolite compounds, one of which is flavonoids which are useful as anti-inflammatory, antioxidant and antibacterial (Roy *et al*., 2022). Based on phytochemical test, torch ginger flower extract has active compounds such as quercetin 42,20 mg/kg; tannin 12,52 mg/kg; saponin 7,05 mg/kg; phenol 5,45 mg/kg and alkaloid 5,25 mg/kg. Quercetin is a flavonoid with various

pharmacological activities, such as anti-inflammatory, antioxidant, antiviral, immunomodulatory and anticancer activity with a low toxicity profile (Aghababaei and Hadidi, 2023). Quercetin can ward off free radicals and transition ions so that quercetin can prevent cancer, chronic inflammation and atherosclerosis. Flavonoids can treat wounds and act as astringents and antimicrobials which can be responsible for wound contraction and increase epithelialization. Flavonoid content can help accelerate collagen growth (synthesize collagen) by increasing fibroblasts and tissue formation (Zulkefli *et al*., 2023). Unlike flavonoids, tannins and saponins are included in the polyphenol group which has an astringent taste and has antioxidant, antibacterial and antifungal activity (Noer *et al*., 2023). Tannins as astringents cause skin pores to shrink, thicken the skin, stop exudate and bleeding so that they can coat wounds. Saponins work by stimulating the formation of new cells and growth factors that cause multiplication, the formation of blood vessel endothelial cells and fibroblasts so that damaged blood vessels can be repaired (Efendi *et al*., 2020). When combined, these compounds may have a synergistic effect that goes beyond their individual contribution to wound healing. The bioactive components in the extract have antimicrobial and antioxidant properties which can stimulate blood coagulation, fight infections and accelerate wound healing (Criollo-Mendoza *et al*., 2023). The antioxidants present in quercetin, phenols, and tannins work together to neutralize free radicals, preventing oxidative damage to tissues at the wound site. Awolola *et al*. (2021) stated that saponin and alkaloid can ward off microbes and act as an anti-inflammatory. These compounds act as natural antibiotic which can help the body to fight infection and microbial invasions.

These various active compounds have antioxidants activity that are beneficial to the body at the right concentration. At high concentrations, these active compounds can act as prooxidants which endanger the wound healing process. High doses of flavonoids can act as mutagens, pro-oxidants that produce free radicals, and as inhibitors of enzymes involved in the metabolism of hormones that are detrimental to the host body (Skibola and Smith, 2000). This condition is not only caused by flavonoids, but other secondary metabolites can also cause similar effects. According to (Thawabteh, 2019), high doses of alkaloids show toxic effects such as paralysis, asphyxia and even death. According to Gavanji *et al*. (2023), adverse skin reactions to herbal remedies can be caused by skin contact or long-term exposure to herbal remedies. This reaction is also related to several risk factors, such as side effects, dosage, health conditions and drug interactions (Adiana and Maulina, 2022).

Antioxidants are chemicals commonly used for topical application and can contribute to fighting radical species that are responsible for much skin damage (Gulcin, 2020). An increase in free radicals without proper effective action of endogenous and exogenous antioxidant systems will produce conditions of oxidative stress, potentially causing skin disorders ranging from functional to even aesthetic disorders, with destruction of structural proteins and cellular changes (Silva *et al*., 2017). The torch ginger flower extract cream with a concentration of 2.5% showed better healing results compared to concentrations of 5% and 10%. The antioxidant content in cream is thought to have an effective effect at low concentrations rather than high concentrations. It is suspected that the active compound content could be antagonistic at greater concentrations. Previous research has reported that pharmacologically, the herbal response is different from standard synthetic drugs because it involves many active compounds that can work on one or several targets, an increase or decrease in these components can have synergistic and antagonistic properties which will affect the effectiveness of therapy (Syahrir *et al*., 2016; Zhou *et al*., 2016).

Topical medicinal preparations can cause side effects in the form of skin irritation where a product is applied. Irritation can be caused by the formulation and/or active substances in the preparation (Sumarni, 2022). The second highest component contained in torch ginger flower extract was tannin (12.52 mg/kg extract). Tannins can accelerate wound healing, re-epithelialization and hair follicle growth in rodents by increasing the expression of growth factors such as *basic fibroblast growth factor* (bFGF), *transforming growth factor-beta* (TGF-β) and VEGF, as well as reducing inflammatory cytokines such as IL-1 and IL-6 and activating the ERK 1/2 pathway (Jing *et al*., 2022).

The high concentration of the torch ginger flower extract cream in this study, namely 5% and 10%, is thought to have a higher tannin content than 2.5%, causing dehydration of wound tissue so that collagen formation is hampered. This is confirmed by the percentage of collagen density in this study which tends to be low on histopathological examination with Masson Trichrome staining. In previous research, the concentration of torch ginger flower extract was higher than that given in this study, namely 20% to 40%. At this high concentration, macroscopic wound closure occurs, however, this research had shortcomings because microscopic observations were not carried out to see the wound healing process (Widyawati *et al*., 2019). Tannin has properties as an astringent which can cause skin pores to shrink, thicken the skin, stop exudates and bleeding so that it can coat wounds (Efendi *et al*., 2020). Furthermore, tannin concentrations that are too high can cause the skin to lose moisture and become dry (Malangngi *et al*., 2012). Dehydrated skin conditions affect collagen formation (Giubertoni *et al*., 2024). Collagen is the main structural material in animal skin and is the main component of the skin which acts as barrier physical relationship between

the environment and body fluids (Haverkamp et al., 2022). In dehydrated skin, the dominant type I collagen can dry out and undergo structural degradation (Giubertoni *et al*., 2024). Fibroblast activity and a decrease in the number of blood vessels will result in the skin losing elasticity and reducing the thickness of the epidermis (Pu *et al*., 2023).

CONCLUSIONS AND RECOMMENDATIONS

The 2,5% torch ginger flower extract cream has skin wound healing activity by reducing inflammatory response, inducing collagen density and down-regulating the expression of IL-6.

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NOVELTY STATEMENTS

Research on the wound healing effects of torch ginger flowers (*Etlingera elatior*) has been widely conducted. However, research that links the activity of the active compounds of torch ginger flowers in the excision wound healing model with inflammatory responses, collagen density and IL-6 expression as parameters is still rarely studied.

AUTHOR'S CONSTRIBUTIONS

Nuralifa Satyabrata conceptualized, managed, performed all the experimental procedures and conducted data analysis and interpretation. Sitarina Widyarini conceptualized, supervised and gave feedback for the study. Yos Adi Prakoso supervised the study, performed the immunohistochemistry procedure and gave feedback for the study. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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