



Ethanol Extract of *Curcuma zedoaria* Enhances Burn Wound Healing in Male White Rats

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Abstract: In addition to its widespread use as a culinary spice, white turmeric rhizome (*Curcuma zedoaria* (Christm.) Roscoe) exhibits important pharmacological activities, including anti-inflammatory, anticancer, and antioxidant effects. The rhizome contains secondary metabolites such as flavonoids, alkaloids, saponins, phenols, tannins, and terpenoids, which are believed to contribute to its potential efficacy in treating burns. This study used a fully randomized post-test-only control group design consisting of five treatment groups: P1 (5% ethanol extract of white turmeric rhizome), P2 (10%), P3 (15%), K⁺ (positive control: Bioplacenton), and K⁻ (negative control: Vaseline flavum). Parameters observed included reduction in burn wound diameter and collagen density, which were assessed through histological analysis. The results showed significant differences between treatment groups ($p < 0.05$). The 10% concentration showed the highest wound healing activity, reaching a healing rate of 54.72% and producing denser collagen compared to the other treatments. However, its effect did not exceed the positive control (Bioplacenton). The 5% concentration showed moderate healing activity (43.84%) with intermediate collagen density, while the 15% concentration produced the lowest effect. Overall, the findings indicate that the ethanol extract of white turmeric rhizome, particularly at a concentration of 10%, enhances burn wound healing and increases collagen formation, supporting its potential as a natural therapeutic agent for the treatment of burns.

Introduction

Burns are a form of tissue trauma that can occur in all age groups and remain a global health problem. The World Health Organization (WHO) reports that approximately 265,000 deaths each year are caused by burns, especially in developing countries (1). Burns are defined as tissue damage or loss due to exposure to extreme temperatures, either hot or cold, which can come from fire, hot liquids, steam, electricity, or radiation (2). In Indonesia, based on the Basic Health Survey (Risksesdas), the prevalence of burns reached 0.7%, with the highest incidence rate in the 1-4 year age group at 1.5% (1).

The skin, the body's largest organ, is the structure most frequently affected by burns. In adults, skin comprises approximately 7% of body weight, with a surface area of 1.5-1.9 m² and a thickness varying between 0.5-4 mm. Heat exposure causes capillary damage and increased vascular permeability, leading to tissue edema and decreased intravascular fluid volume. These pathophysiological effects vary with the depth of the injury, ranging from excessive evaporative fluid loss in first-degree burns to tissue fluid retention in second-degree burns, to eschar formation and exudation in third-degree burns (3).

Burn wound management generally involves topical

therapy to control inflammation, prevent infection, and accelerate tissue regeneration. Commonly used pharmaceutical agents include silver sulfadiazine, bacitracin, and bioplacenta. While effective, these drugs have limitations, particularly in terms of cost and potential long-term side effects, prompting the search for more affordable and safe natural-based alternatives (4, 5).

One of the herbal plants that has the potential to be developed as a burn therapy agent is White Turmeric (*Curcuma zedoaria* (Christm.) Roscoe). This plant is traditionally used as a cooking spice, a supplement for postpartum mothers, and to treat digestive disorders and fever (6). Several studies have shown that *C. zedoaria* has biological activities relevant to the wound healing process, including antioxidant and anti-inflammatory effects. The diferuloylmethane content in its rhizomes has been reported to delay the aging process and degenerative diseases at a dose of 20 mg/mL through a free radical scavenging mechanism (7). Furthermore, a study by Rahman *et al.* (2021) showed that *C. zedoaria* rhizome extract exhibited maximum anti-inflammatory activity 2-6 h after administration (8).

Chemically, *Curcuma zedoaria* rhizome contains various secondary metabolites that contribute to its pharmacological activity, including terpenoids (especially sesquiterpenoids

and monoterpenoids), flavonoids, phenolics, tannins, saponins, alkaloids, and steroids (4, 9). Flavonoids and phenolic compounds act as powerful antioxidants that can capture free radicals, thereby protecting cells from oxidative damage and supporting the proliferation phase of wound healing. Terpenoids, especially sesquiterpenoids such as curcumenol and curcumenone, are known to have anti-inflammatory effects through inhibition of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, which play a role in arachidonic acid metabolism and the formation of inflammatory mediators. This mechanism is important in suppressing increased capillary permeability and exudate formation in burns (10).

In addition, alkaloids in *C. zedoaria* have antimicrobial and astringent activities, which can prevent secondary infections and accelerate new tissue formation by strengthening collagen fibers and supporting DNA synthesis (11). GC-MS analysis of *C. zedoaria* essential oil also identified β -tumerone, 1,8 - cineole, and zingiberene as the main constituents contributing to the anti-inflammatory and wound healing effects (12).

Although various studies have reported the pharmacological activity of *Curcuma zedoaria*, research specifically evaluating the potential of White Turmeric rhizome as an anti-burn agent, particularly in topical preparations and related to the biological mechanisms of wound healing, is still limited. Therefore, further studies are needed to assess the effectiveness of *Curcuma zedoaria* as a natural-based burn therapy agent, with a mechanistic approach that supports the hypothesis of its use in accelerating burn healing.

Methodology or Experimental Section

Materials

The materials used in this study included fresh white turmeric rhizomes (*Curcuma zedoaria*), solvents and reagents for the extraction process and phytochemical screening (ethanol of various concentrations, distilled water, HCl, H_2SO_4 , chloroform, anhydrous acetic acid, Mayer and Dragendorff reagents, Mg, gelatin, and NaCl), as well as materials for the manufacture of topical preparations. For *in vivo* testing, experimental animals were used along with their food and drink, anesthetic and depilatories (Veet®), comparison agents (Bioplacenton®), and histological materials in the form of formalin buffer, xylene, paraffin, and hematoxylin-eosin (HE) stain.

The main equipment used includes preparation tools for simple drugs and extracts, laboratory glassware, rotary evaporators, analytical scales and animal scales, ovens, water baths, and devices for making and evaluating ointment preparations. In addition, surgical equipment and animal care equipment are used, as well as histopathology equipment such as microtomes and light microscopes for tissue observation.

In Vivo Test

Animal Preparation

The determination of the minimum number of experimental animals is based on the Federer formula, namely $(t-1)(n-1) \geq 15$ where t is the number of treatments and n is the number of repetitions of each treatment, so this study used 5 mice per group. In this study, 25 white mice weighing 200 – 250 grams aged 75 – 90 days were needed because cell

proliferation at this growth age is fast, thus supporting the wound healing process.

The experimental animals were first acclimatized for 7 days to allow them to adapt to the environment. During this period, their general condition was observed and their body weight was measured. Weight measurements were taken to assess weight gain during the treatment. If the change in body weight is no more than 10%, it indicates that the experimental animal is in good health and can be included in the study.

Sample Administration

The White Turmeric (*Curcuma zedoaria*) rhizome used in this study was obtained from the Spice and Medicinal Plants Research Institute, Bogor, West Java. Further sample determination and identification were carried out at the Plant Taxonomy Laboratory, Padjadjaran University.

Extraction

The rhizomes of *Curcuma zedoaria* were first sorted, washed, and dried in an oven at 50°C. The dried rhizomes were then ground using a blender and sieved through a 60-mesh sieve. The extract was prepared using a maceration method by soaking the turmeric rhizomes in a dark tube and pouring 70% ethanol with a 1:1 ratio of the drug to the solvent for 3x24 h. The maceration results were then concentrated using a rotary evaporator to obtain a thick extract.

Characterization Test

This testing includes specific characteristics, including sample identity and organoleptic identification, as well as non-specific characteristics, including moisture and ash content. Phytochemical screening is also performed.

Research Design

The research design used was a completely randomized design (CRD) with a post-test only control group approach. The experimental animals were divided into five groups (K^+ , K^- , P_1 , P_2 , P_3), with each group consisting of five white rats. The treatment groups (P_1 , P_2 , P_3) received ethanol extract of *Curcuma zedoaria* rhizome with concentrations of 5%, 10%, and 15%, respectively. Positive control (K^+) was given topical treatment with bioplacenta ointment and negative control (K^-) was given topical treatment with yellow vaseline.

Burn

The experimental white mice were initially weighed and then divided into five groups of five mice each. The burn area was determined on the back of each mouse. A mat is placed under the animal, and gloves are worn during the procedure. The fur on the backs of the mice was shaved, followed by the use of Veet® for a more thorough shave, and the burn area was marked with a marker. General anesthesia was induced using 0.25 mL of Castran®. The designated skin area was disinfected with alcohol and allowed to dry. A metal rod was heated over a blue flame for 3 min and then applied to the skin of the backs of the anesthetized mice for 5 s to induce a burn. The resulting wound was compressed with gauze soaked in distilled water for 1 minute, after which the diameter of the burn was measured.

Observation of Wound Healing Process

Each mouse in the control and treatment groups received the ointment twice daily for 14 days. Observations were made over the 14 days by measuring the diameter of the

burn zone using a caliper with a 0.01 mm scale. Healed burns were indicated by wound closure.

Variables

The independent variable is the variation in the concentration of turmeric extract, while the dependent variables include the diameter of the burn wound on the back of white mice during the observation period, collagen distribution, and the formation or regeneration of epithelial cells.

Histological Sampling

Skin biopsy samples were taken on day 15, with each sample covering the wound area by taking a section of muscle tissue and subcutaneous fat from each animal. Tissue was removed using a scalpel blade that had been cleaned with ethanol. Prior to sampling, the experimental animals were anesthetized using an overdose of castran. Skin samples were immediately immersed in 10% neutral buffered formalin.

Histological Preparation and Observation

The wound tissue was excised transversely and fixed using 10% formalin. The tissue was then dehydrated gradually with 70% alcohol for 1 hour, 80% alcohol for 1 hour, 90% alcohol for 1 hour, 96% alcohol for 1 hour, absolute ethanol I for 1 hour, and absolute ethanol II for 1 hour. Tissue cleaning was carried out with xylene for 1 hour to remove residual alcohol. The printing process used paraffin so that it was printed on a paraffin block, then placed on a cooling plate. The paraffin block was cut into thin 3-5 μm using a microtome. The sections are stained with hematoxylin and eosin to obtain preparations that can be observed under a light microscope.

Observations of collagen distribution and cell formation or regeneration were performed using a light microscope (Olympus). The preparations were observed at 40 \times 10 magnification in one field of view to determine collagen density, indicating the treatment's highest effectiveness, as indicated by the density of collagen formed.

Data analysis

Data on the content of secondary metabolites in White Turmeric extract were observed descriptively, data on the diameter of the burn wound were analyzed using IBM SPSS Statistics 21. Testing was carried out using One Way Anova with a 95% confidence level to determine the average difference between treatment groups and then continued with Duncan's advanced test to see significant differences between groups. Meanwhile, data on collagen distribution were analyzed using qualitative descriptive.

Results and Discussion

Determination, Rendement and Phytochemical Screening

In this study, White Turmeric rhizome (*Curcuma zedoaria* (Christm.) Roscoe) was obtained from the Spice and Herbal Medicine Research Institute, Bogor Agricultural University, West Java. Prior to further procedures, plant identification was performed to confirm the identity of the materials used, ensuring the accuracy and reliability of the research. The identification process was conducted at the Plant Taxonomy Laboratory, Department of Biology, Padjadjaran University. For the preparation of simplicia, rhizomes are the plant parts used. The process begins with the collection of fresh

rhizomes, followed by wet sorting and slicing.

After slicing, the samples were weighed, resulting in a total weight of 4 kg. The next stage involved drying the samples in an oven at 50°C. In accordance with the findings of Manalu and Adinegoro (13), optimal drying conditions were achieved when the dried simplicia contained less than 10% moisture, showed minimal shrinkage, and maintained good visual quality. After drying, the samples underwent dry sorting and were ground using a grinder, resulting in a final weight of 454 g. Based on these results, the yield of turmeric rhizome simplicia powder was calculated to be 11.32%.

Extraction was carried out using 454 grams of white turmeric rhizome powder macerated in 70% ethanol. Ethanol was chosen as the solvent due to its ability to dissolve both polar and non-polar compounds, as well as its safety for researchers. Maceration was carried out for 3x24 h in the hope that the solvent would maximally extract the secondary metabolites contained in the turmeric rhizome. The resulting maceration yielded 3 liters, followed by evaporation using a rotary evaporator at 60°C to separate the extract from the solvent. The resulting thick extract was 133 grams. The yield percentage was 29.30%, which is in accordance with the Indonesian Herbal Pharmacopoeia, which states that the yield of thick turmeric rhizome extract should not be less than 17.6% (14).

The water content obtained was 13.15%, indicating that it is still within the standard range and in accordance with the Indonesian Herbal Pharmacopoeia standards, which require the water content in thick extracts not to exceed 14.0%. The ash content obtained was 1.97%, indicating that it is also still within the standard limit of less than 8.6% (14). Based on the results of phytochemical screening, it was found that the White turmeric rhizome extract contains alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids.

Observation Results of Burn Wound Healing

Furthermore, burn induction was carried out where the wounds selected in this study were second-degree burns because first-degree burns can heal faster and can heal without treatment, while third-degree burns have a high level of severity so they require a longer healing time and also require special therapy. Based on the measurement of the diameter of the burn wound for 14 days, the following results were obtained.

Figure 1 visually shows that burn wounds for 14 days showed that all groups receiving white turmeric rhizome extract (*Curcuma zedoaria*) treatment experienced improvement in wound appearance compared to negative controls. The wound area in the treatment group showed progressive shrinkage, accompanied by a smoother and healthier colored tissue surface, indicating tissue regeneration and epithelialization.

Among the treatment groups, the 10% concentration extract (P2) showed the best visual response, characterized by faster shrinkage of the wound area, a flatter epithelial surface, and minimal signs of residual inflammation. In contrast, wounds in the negative control group (Vaseline flatum) appeared wider, non-uniform, and showed slow healing, reflecting the limitations of Vaseline as a protective agent without active biological activity.

The positive control group (Bioplacenton®) showed the most advanced level of healing with almost complete wound closure, thus serving as a clinical benchmark for the effectiveness of burn wound healing. Qualitatively, these

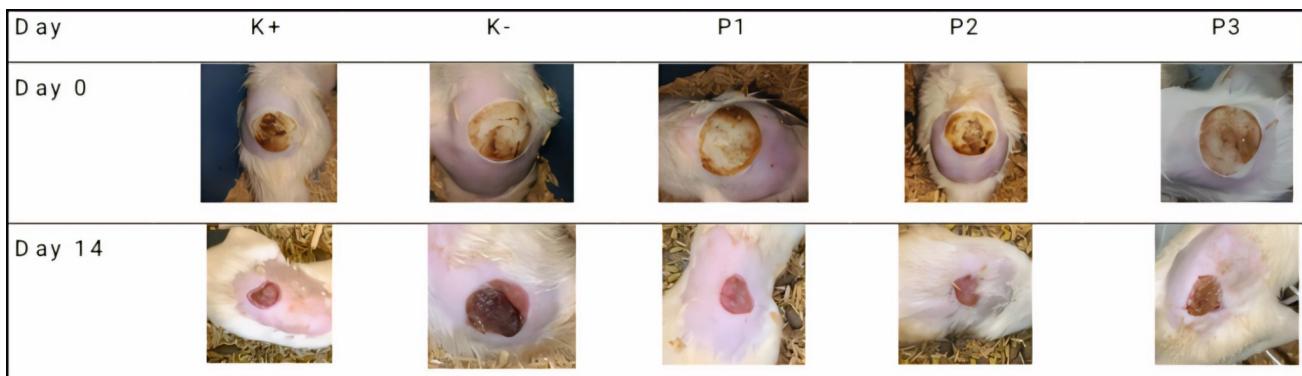


Figure 1. Result of burn healing (K+ = positive control, K- = negative control, P1 = concentration of 5%, P2 = concentration of 10%, P3 = concentration of 15%).

findings indicate that white turmeric rhizome extract, particularly at a concentration of 10%, has real potential in accelerating the healing of second-degree burns.

Table 1 presents the changes in burn diameter and percentage of healing during 14 days of observation. Quantitatively, all treatment groups showed a decrease in wound diameter compared to the negative control, which indicates the occurrence of wound contraction and tissue regeneration. The greatest and most consistent reduction in wound diameter was observed in the group given ethanol extract of white turmeric rhizome (*Curcuma zedoaria*) at a concentration of 10% (P2). These patterns are further illustrated in **Figure 2**, which summarizes the percentage of burn wound healing across all experimental groups.

These results are reflected in the percentage of wound healing, where the P2 group achieved the highest value among the treatment groups and approached the positive control (Bioplacenton®). In contrast, group P1 (5%) showed slower healing, while group P3 (15%) showed decreased effectiveness. This pattern indicates a nonlinear dose-response relationship, where intermediate

concentrations optimal therapeutic effects, while further increases in concentration do not improve wound healing effectiveness.

The negative control group (Vaseline flavum) maintained a larger wound diameter throughout the observation period as well as the lowest healing percentage, confirming that Vaseline only acts as a passive barrier without sufficient biological activity to stimulate tissue repair.

The graphical representation shows that all extract concentrations produced an observable healing response compared with the negative control. Among the three extract concentrations, the 10% group (P2) produced the greatest improvement, achieving a mean healing percentage of 54.72%, which closely approached the performance of the positive control treated with Bioplacenton (56.88%).

The 5% concentration (P1) demonstrated moderate healing activity with a mean healing rate of 43.84%, whereas the 15% concentration (P3) yielded the lowest efficacy among the extract groups. The negative control group, treated with vaseline flavum, exhibited the smallest percentage of wound contraction (21.04%). These findings confirm that the ethanol extract of white turmeric rhizome significantly promotes burn wound healing, with the optimal effect occurring at the 10% concentration.

Based on the results of the statistical analysis using one-way analysis of variance (One-Way ANOVA), treatment with white turmeric (*Curcuma zedoaria*) rhizome extract showed a significant effect ($p < 0.005$) on the average burn wound measurements. The graph indicates that all treatment groups exhibited an influence on burn healing; however, the most effective treatment was Group 2, which received a 10% concentration of white turmeric rhizome ethanol extract. This group achieved a healing percentage of 54.72%, approaching that of the positive control group treated with Bioplacenton, which showed a healing percentage of 56.88%. In contrast, the negative control group treated with Vaseline flavum exhibited the lowest healing rate at 21.04%. These results demonstrate that the 10% concentration of white turmeric rhizome extract provides optimal burn healing activity compared to other tested concentrations.

Table 1. Results of of healing of measuring the diameter and percentage burns.

Treatment Group	AUC Diameter \pm SEM	Cure Percentage (%)
Positive control	26.147 ± 0.62^a	58.96%
Negative control	32.139 ± 0.30^c	21.04%
Treatment 1	30.784 ± 0.49^{bc}	43.84%
Treatment 2	29.794 ± 0.71^b	54.72%
Treatment 3	31.565 ± 0.35^c	36.88%

Notes: Superscript information with different lowercase letters on the same line shows a significant difference $p < 0.05$.

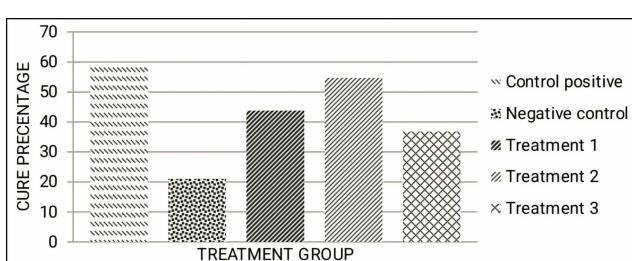


Figure 2. Diagram illustrating the percentage of burn wound healing.

Histological Results

Histological observations of collagen fibers in skin tissue were conducted on day 15, corresponding to the proliferative phase, during which new tissue (granulation tissue) formation occurs. Collagen synthesis is important in the wound healing process. When tissue is damaged, collagen

plays a role in restoring anatomical structure and function. Collagen synthesis in the proliferative phase can be maximized if the inflammatory period is not prolonged (15). The histological differences in collagen density among treatment groups are presented in **Figure 3**.

The results of histological observations of skin tissue showed that the positive control group (Bioplacenton®) had the highest collagen density, characterized by thick, dense, and neatly arranged collagen fibers. These findings are consistent with the mechanism of action of Bioplacenton® which combines placental extract and neomycin sulfate. Placenta extract is known to contain biogenic stimulators that can increase cell metabolism, stimulate tissue regeneration, and accelerate wound healing, as proven in various *in vivo* and *in vitro* studies. Meanwhile, neomycin sulfate acts as a broad-spectrum antibacterial agent that prevents secondary infections, thereby creating an optimal environment for collagen deposition (16).

In contrast, the negative control group showed no significant collagen formation, indicating inhibition of the proliferative phase of wound healing. This underscores the importance of pharmacological interventions to support fibroblast migration and collagen synthesis after burns.

In the treatment group with White Turmeric (*Curcuma*

zedoaria) rhizome extract, an increase in collagen density was seen which varied between concentrations. Treatment P2 showed the best results, with collagen fibers starting to thicken and become more tightly arranged compared to P1 and P3, although there was still some space between the fibers. These findings indicate a dose-dependent response, where intermediate concentrations provide the most optimal effect on wound healing. This activity can be mechanistically explained through the content of flavonoids and phenolic compounds which act as antioxidants, reducing oxidative stress at the wound site, as well as terpenoids which have anti-inflammatory effects through inhibition of the COX and LOX pathways. This combination of effects supports fibroblast activation and collagen synthesis in the proliferative phase of wound healing.

Interestingly, increasing the extract concentration up to P3 did not result in higher collagen density compared to P2, and even tended to show a decrease in the quality of the collagen tissue. This phenomenon indicates an inhibitory effect at high doses, which may be due to the mild cytotoxic effects of certain bioactive compounds or increased viscosity of topical preparations, thus inhibiting the diffusion of oxygen and nutrients to the wound tissue. Oxygenation is a crucial factor in wound healing, especially in the proline and

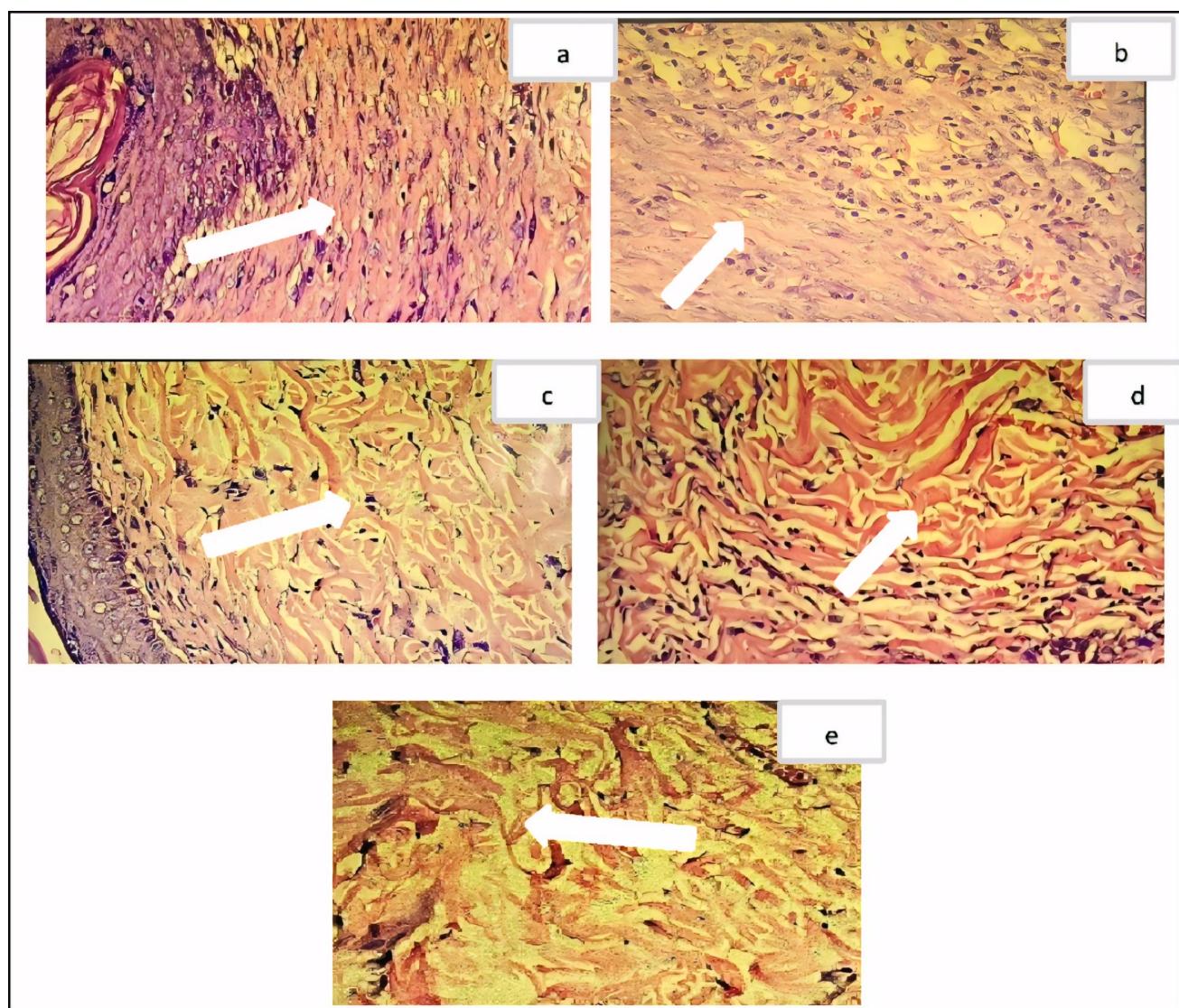


Figure 3. Collagen thickness in each treatment group (Hematoxylin-Eosin staining, magnification 400x): (a) positive control group, (b) negative control group, (c) treatment group 1, (d) treatment group 2, and (e) treatment group 3. White arrows indicate collagen fibers.

lysine hydroxylation processes required for collagen fiber stability (17). Thus, despite higher active substance content, its biological effectiveness may be reduced due to the limitations of the wound microenvironment.

Treatment P1 (5% concentration) has a higher moisture content compared to P2 (10% concentration) and P3 (15% concentration) which are derived from vaseline flavum, where the dominant oil properties of vaseline flavum can maintain skin moisture and prolong drug contact with the skin, as well as increase percutaneous absorption of the drug's active substance (18).

The moisture level of the ointment differed between treatments. P1 (5%) had the highest moisture content due to its vaseline flavum content, while P3 (15%) showed lower moisture content compared to P1 and P2 (10%). This difference is in line with the histological results: the collagen density of P2 was higher than that of P3 even though P3 had a higher extract concentration, indicating a paradoxical effect. The paradoxical effect in P3 was caused by a combination of the concentration of the active substance and the physical properties of the ointment, including adhesion and distribution of the extract, which decreased the effectiveness of wound healing.

In some types of drugs, high doses cause the direct release of histamine from mast cells, causing blood vessels to become more permeable to plasma fluid and causing an inflammatory process (19). In addition, the more extract used in a preparation, the lower the viscosity of the preparation, which affects the adhesive power of the extract (20).

In the group with a concentration of 15%, adhesion was not as good as ointment with a concentration of 5% and 10% so that the therapeutic effect was not achieved (21). In the negative control group (ointment base) produced the lowest collagen density among all treatment groups, this occurred because group K was a group of mice that were given burn treatment without effective substances or medicinal ingredients to help the wound healing process, resulting in a longer inflammatory period (22).

Ethanol extract of *Curcuma zedoaria* rhizome contains flavonoids, alkaloids, phenols, saponins, tannins, and terpenoids that support wound healing through specific mechanisms; flavonoids protect cells from oxidative stress (23), alkaloids are antimicrobial and accelerate the growth of new tissue (11, 24), phenols scavenge free radicals thereby suppressing inflammation (25), saponins stimulate the proliferation of macrophages and fibroblasts for collagen deposition, and terpenoids accelerate wound contraction and angiogenesis (26, 27). Histological results showed the highest collagen density in the positive control (Bioplacenton) (16), followed by P2 (10%), P1 (5%), and P3 (15%); although P3 had the highest concentration, collagen density decreased (paradoxical effect) due to decreased moisture and ointment adhesion which reduced tissue oxygenation and penetration of active metabolites so that collagen synthesis was inhibited (17-21). P2 showed an optimal balance between the amount of active metabolites and tissue conditions, resulting in the best collagen thickening, while P1 continued to support collagen through high humidity, and the negative control showed the thinnest collagen due to the absence of active substances that stimulate healing (22).

Conclusion

Based on the results of this research on the effectiveness of the ethanol extract of the *Curcuma zedoaria* ethanol extract significantly increased burn healing in male white rats, indicated by a wound closure percentage of 54.72% and higher collagen density compared to 5% and 15%, although still lower than Bioplacenton (56.88%) (16, 17, 22). The 10% concentration produced an optimal balance of active metabolites and tissue conditions, while 15% decreased effectiveness due to decreased moisture and ointment adhesion. This study has limitations in the form of a small sample size, did not use biochemical markers such as hydroxyproline, and did not evaluate the dose response beyond the topical concentration tested.

Abbreviations

AUC = Area Under the Curve; SEM = Standard Error of the Mean.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The data supporting the findings of this study are available

from the corresponding author upon reasonable request.

Ethics Statement

All animal experiments were approved by the Research Ethics Committee of Universitas Andalas (Approval No. 160/UN.116.2KEP-FK/2023) and conducted in accordance with relevant guidelines and regulations.

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Additional Information

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