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IN VIVO MACROSCOPIC STUDY OF ZIZIPHUS JUJUBA EXTRACT OINTMENT IN ACCELERATED BURN WOUND HEALING IN RATS

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ABSTRACT

Burns are type of injury that damage the skin which caused by exposure to heat sources that requiring comprehensive management and potentially leading to high morbidity. Limited resources and costs often encourage the use of herbal ingridients as an alternative. Ziziphus jujuba (Chinese date) has been traditionally used in Chinese medicine (TCM) for its healing property. Ziziphus jujuba contains various bioactive compounds such as flavonoids, saponins, and tannins that contribute to wound healing through their anti-inflammatory, antioxidant and regenerative mechanisms. This study aims to test the effectiveness of Ziziphus jujuba extract ointment on burn wound healing in Rattus norvegicus. Ointment preparations were made in concentrations of 10 mg/mL and 20 mg/mL, then tested physically (organoleptic, homogeneity, pH, spreadability). This study used a true-experimental method with a post-test only control group design, involving four treatment groups, each consisting of six rats with back burns: control (K), MEBO as control positive (P1), 10 mg/mL Ziziphus jujuba extract ointment (P2), and 20 mg/mL Ziziphus jujuba extract ointment (P3). The study was conducted 21-day obervation period to evaluate wound diameter, wound healing percentage, macroscopic and microscopic assesment based on the modified Nagaoka criteria. The group treated with Ziziphus jujuba ointment 20 mg/mL (P3) achieved the best healing outcomes, chracterized by faster wound reduction, minimal adverse local and allergic reactions, and shorter healing period than those observed in the control and other treatment groups.

KEYWORDS *Burns, Ziziphus jujuba, Ointment, Wound Healing, Rattus Norvegicus.*

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INTRODUCTION

Burns are a type of injury to the skin resulting from direct or indirect exposure to heat sources. The incidence and prevalence of burns are quite high, thus increasing the risk of morbidity and mortality significantly. (Ministry of Health of the Republic of Indonesia, 2020).

According to the World Health Organization (WHO), there are about 90% of burn cases occurring in countries with low socioeconomic status, especially in developing countries. Data from WHO shows that the highest incidence of burns is in women in the Southeast Asian region. Of the 27% of total deaths from burns globally, almost 70% occur in women. National data on the death rate and incidence of burns in Indonesia is still not available. (Ministry of Health of the Republic of Indonesia, 2020)

From the results of an epidemiological study conducted at Cipto Mangunkusumo Hospital (RSCM) in 2011-2012, it was recorded that 303 were treated for a period of 2 years. Of the 303 patients, 45.87% had severe burns with an area of 20-50%. Outside Jakarta, data from Sanglah General Hospital Denpasar in 2012 recorded as many as 154 patients treated, 8.42% (13 people) of whom died due to fire explosions that caused extensive and deep burns. Meanwhile, at Sardjito Hospital Yogyakarta in 2012, which was the second year of the eruption of Mount Merapi, there were 49 patients treated in the burn unit. Of this total, 53.3% (16 patients) died. (Ministry of Health of the Republic of Indonesia, 2020).

Treatment of burns must be comprehensive, consisting of first aid efforts by cooling the wound, cleaning the wound, administering intravenous fluids, local treatment with antibiotics to prevent infection, long-term recovery to surgical reconstruction of more severe wounds. All of these actions require quite a lot of resources, both from medical equipment, health workers, and medicines, so that the cost for burn treatment becomes very high. (Medecins Sans Frontieres, 2022).

In Indonesia, burn treatment centers are still very limited. Due to the limited resources and high cost for burn treatment, there is often the use of natural herbal ingredients for burn healing. One of the herbal ingredients that has the potential for this is *Ziziphus jujuba*, which is often called red dates or Chinese dates. *Ziziphus jujuba* is known to contain many nutrients, so it is often used in traditional Chinese medicine (TCM). (Agrawal et al., 2023). (Ministry of Health of the Republic of Indonesia, 2020).

From the results of the research conducted by Batovska et al (2024), show that *Ziziphus jujuba* has many benefits for the skin and hair, one of which is the benefits for the wound healing process. The anti-inflammatory, antiviral, antifungal, and antibacterial properties possessed by jujuba are related to its rich composition of fatty acids, *β*-carotene, α-tocopherol, phenolic compounds, vitamins A, E, C, tannins, cyclopeptides, cfeic acids, and flavonoids. These components play a role in improving wound healing ability in *Ziziphus jujuba* by encouraging collagen formation and tissue epithelialization processes. (Batovska et al., 2024).

The benefit of this research for further researchers is to provide knowledge about the content, function, and mechanism of action of *Ziziphus jujuba* extract, which can be an opportunity for the development of extracts of natural ingredients and other medicines,

while for the community, this research is expected to be beneficial for the development of natural and safe alternative treatments for burn patients. Thus, this study aims to evaluate the effectiveness of the use of ointments containing *Ziziphus jujuba* extract in the healing process of burn wounds and compare the speed of wound healing with the ointment and other topical treatments in rats (*Rattus norvegicus*).

RESEARCH METHOD

This study is true-experimental with a post-test only control group design which aims to assess the effectiveness of *Ziziphus jujuba* extract ointment on burn wound healing in rats (*Rattus norvegicus*). The experiment was carried out at the Integrated Laboratory of FKKGIK Universitas Prima Indonesia during January-May 2025.

The *Ziziphus jujuba* extract ointment was prepared using dried *Ziziphus jujuba* fruits obtained from a traditional medicine store in Medan. The fruits were re-dried, and selected based on ripeness and quality, deseeded, and ground into powder, then extracted by two-stage maceration using 96% ethanol (1:10). The combined filtrates were evaporated at 50°C to obtain a concentrated extract, which was susquently screened for phytochemicals such as alkaloids, flavonoids, steroids/terpenoids, saponins, and tannins. The ointment base was formulated according to Agoes (2006) using 15 g adeps lanae and 85 g vaselin album, which were mixed in a mortar until homogeneous. The concentrated extract was gradually incorporated into the base to produce extract ointments at concentrations of 10 mg/mL and 20 mg/mL. The formulated ointments were then subjected to a physical evaluations, including organoleptic assessment, homogeneity, pH measurement, and spreadability, to ensure that the preparations met standard topical formulation requirements.

A total of 24 rats (*Rattus norvegicus*) were acclimatized for one week before burn wounds were induced on the dorsal area. The animals were then divided into four groups: the negative control group (KN) received aquadest, the positive control group (KP) was treated MEBO ointment (20 g), the first treatment group (P1) received *Ziziphus jujuba* extract ointment at a concentration of 10 mg/mL, and the second treatment group received *Ziziphus jujuba* extract ointment at 20 mg/mL. All treatments were applied topically twice daily. The study was conducted 21-day period to evaluate wound diameter, wound healing percentage, and macroscopic evaluation based on the modified Nagaoka criteria includes three parameters: wound healing time, local infection, and allergic reaction. (Prasetya & Suhaymi, 2020). The obtain data were analyzed using SPSS with independent variables in the form of *Ziziphus jujuba* extract ointment (10 mg/ mL and 20 mg/ mL) and MEBO, while the dependent variable was the rate of burn healing.

RESULT AND DISCUSSION

The results of phytochemical screening on *Ziziphus jujuba* extract, as presented in Figure 1, showed positive reactions for all tested secondary metabolites, including alkaloids, flavonoids, steroids/terpenoids, saponins, and tannins. These bioactive compounds support the extract's anti-inflammatory and wound-healing potential in burn wound management.

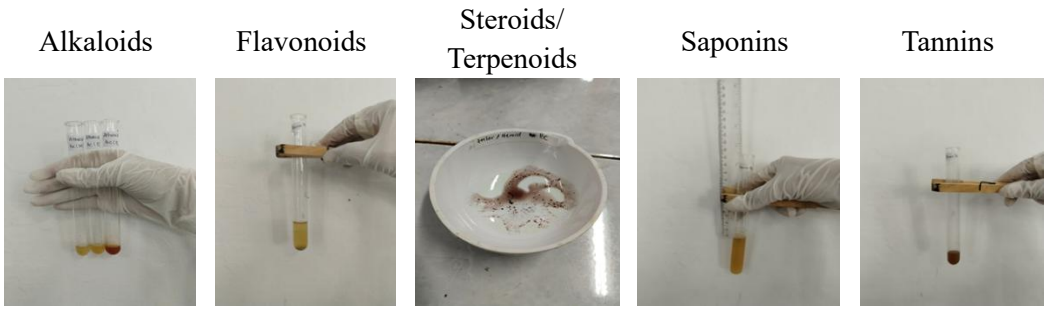
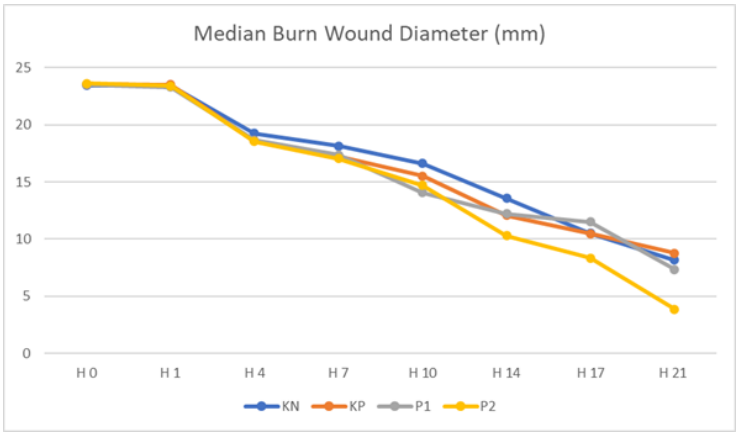


Figure 1. Phytochemical Screening Result of *Ziziphus jujuba* Extract

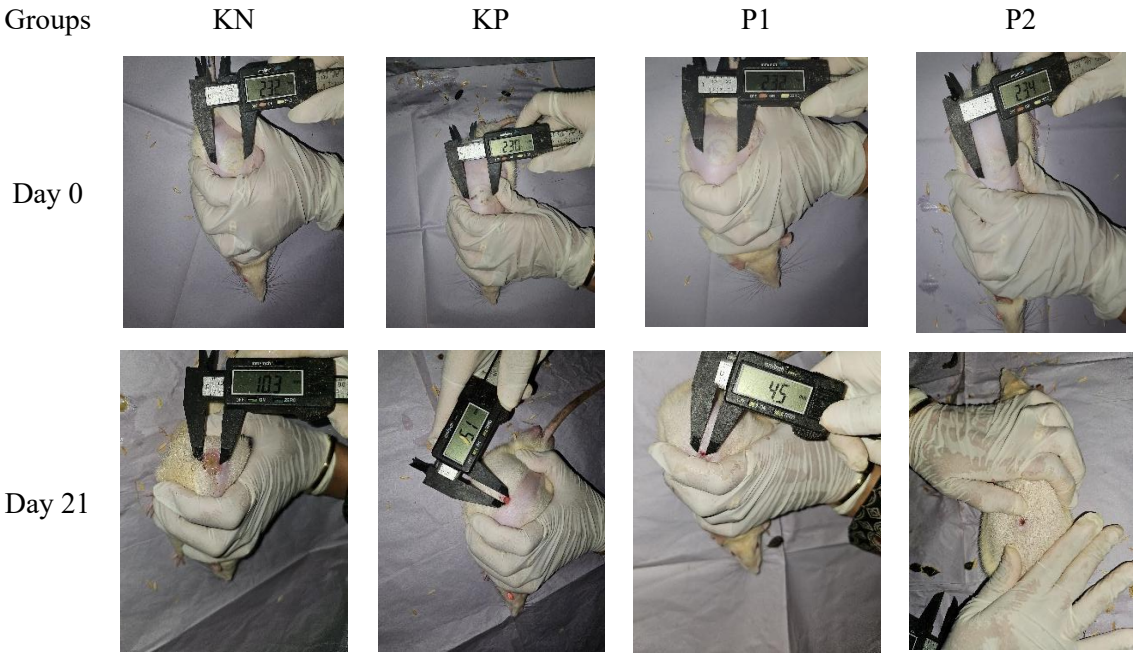
Physical evaluation of *Ziziphus jujuba* extract ointment aims to ensure that the ointment preparations made are of good quality, safe to use, remain stable during storage and are able to provide the therapeutic effect as expected. The physical evaluation of *Ziziphus jujuba* extract ointment showed that both concentrations (10 mg/ mL and 20 mg/ mL) exhibited smooth texture, chocolate to dark-chocolate coloration, and a characteristic odor of the extract. Both formulations were homogeneous, with pH values of 6.11 and 6.05, respectively, which fall within the acceptable range for topical preparations. The spreadability test demonstrated adequate dispersion, with the 10 mg/mL ointment showing a spreadability of 3.83 cm and the 20 mg/mL formulation showing 3.16 cm, indicating good consistency and suitability for topical application.

This study presents the efficacy of *Ziziphus jujuba* extract ointment in accelerating of wound healing in rats, based on several parameters included changes in burn wound diameter, the percentage of wound healing, and macroscopic evaluation based on the modified Nagaoka criteria. The effect of the extract ointment on burn wound diameter was observed by measuring the reduction in wound size across treatment groups.



Notes: KN= negative control group (aquadest); KP= positive control group (MEBO ointment 20gr); P1= Ziziphus jujuba extract ointment 10mg/mL; P2= Ziziphus jujuba extract ointment 20 mg/mL.

Figure 2. Median Burn Wounds Diameter Changes in Each Treatment Group



Notes: KN= negative control group (aquadest); KP= positive control group (MEBO ointment 20gr); P1= Ziziphus jujuba extract ointment 10mg/mL; P2= Ziziphus jujuba extract ointment 20 mg/mL.

Figure 3. Burn Wound Diameter Changes in Each Treatment Group

Based on Figure 2 and 3, showing *Ziziphus jujuba* extract ointment 20 mg/ mL demonstrated the fastest and most pronounced reduction in burn wound diameter compared with the negative control, positive control, and the *Ziziphus jujuba* extract ointment 10 mg/mL. Although, there was no statistically significant

difference in burn wound diameter among groups ($p > 0.05$), the *Ziziphus jujuba* extract ointment 20 mg/ mL group exhibiting the smallest wound diameter, followed by P1, the negative control group, and positive control group , which had the largest remaining wound diameter (9,25; 12,59; 13,92; 14,33). This order indicates that the 20 mg/mL *Ziziphus jujuba* extract ointment provided the most effective contraction of burn wounds. Although the differences were not statistically significant, these findings indicate a meaningful biological effect of the extract in accelerating the burn wound healing process.

The percentage of burn healing was assessed by the formula for calculating the decrease in burn wound diameter during the observation period. The analysis showed that all groups exhibited normally distributed data, as indicated by the *Shapiro–Wilk* test ($p > 0.05$). *Ziziphus jujuba* extract ointment 20 mg/ mL demonstrated the highest mean healing percentage (80.30 ± 22.36), followed by the 10 mg/ mL *Ziziphus jujuba* extract ointment (71.25 ± 25.72), while the negative control (KN) and positive control group (KP) showed lower healing percentages of (63.23 ± 7.83 and 62.66 ± 11.48), respectively. These findings indicate a trend in which higher concentrations of Ziziphus jujuba extract were associated with greater wound contraction compared to both control groups.

Macroscopic evaluation using Modified Nagaoka Macroscopic Criteria showing that all groups wound healing times exceeding 14 days and no allergic reactions detected. However, the control negative group (KN) showed the highest frequency of local infection, while P1 and P2 groups showed an increased numbers of without infection. The *Linear-by-Linear Association* test revealed a significant relationship ($p = 0.007$), indicating that higher treatment concentrations reduced local infection rates.

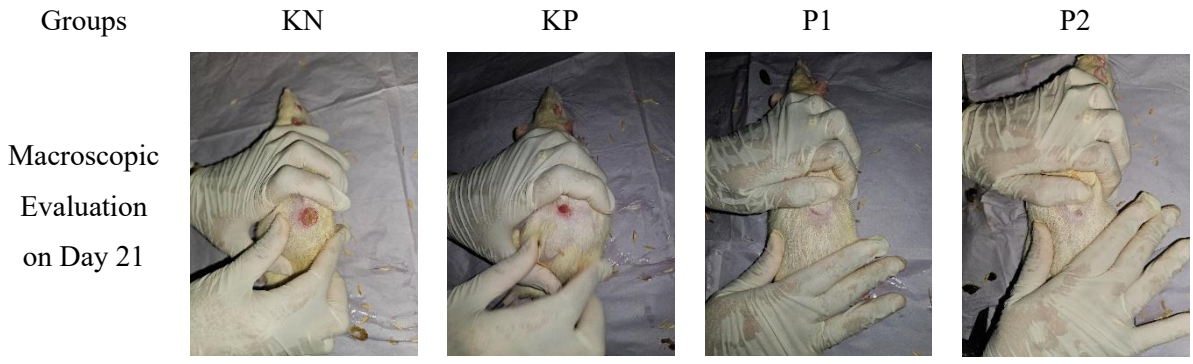


Figure 4. Burn Wound Evaluation Based on Modified Nagaoka Macroscopic Criteria

The results of the study showed that the administration of *Ziziphus jujuba* extract ointment had an effect on accelerating the healing of burns in rats (*Rattus*

norvegicus). The highest average wound healing percentage was obtained in *Ziziphus jujuba* extract ointment 20 mg/ mL demonstrated the highest mean healing percentage (80.30 ± 22.36), followed by the 10 mg/ mL *Ziziphus jujuba* extract ointment (71.25 ± 25.72), while the negative control (KN) and positive control group (KP) showed lower healing percentages of (63.23 ± 7.83 and 62.66 ± 11.48), respectively. These findings indicate that higher extraxt concentrations are associated with improved wound healing outcomes and reduce infection rates in the wound area.

This effect may be explained by the presence of potent bioactive components in *Ziziphus jujuba*, including phenolic acids, flavonoids, triterpenoids, and saponins. These molecules exhibit well-established antioxidant, antimicrobial, and anti-inflammatory properties, which collectively enhance wound healing by reducing infection, modulating inflammation, and stimulating collagen synthesis. This finding is consistent with the study reported by (Hovaneț et al., 2022) who demonstrated that ointments containing *Ziziphus jujuba* leaf extract exhibited significant wound-healing and anti-inflammatory activities, primarily attributed to their high rutin, quercetin, and chlorogenic acid contents. Their study also showed increased collagen production and faster wound contraction in a rat model.

In terms of antimicrobial effects, *Ziziphus jujuba* has been shown to possess activity against a broad spectrum of clinically relevant bacteria frequently implicated in wound contamination. Previous findings by (Batovska et al., 2024) demonstrate that extracts and isolated flavonoid fractions exert inhibitory effects against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus pumilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Raoultella ornithinolytica*, and *Salmonella typhi*, as well as fungal pathogens such as *Candida albicans* and *Aspergillus niger*. The study also reported that flavonoids isolated from *Z. jujuba*, particularly quercetin and hyperin, exhibited targeted antimicrobial activity against *S. aureus* and *E. coli*, with markedly enhanced potency under acidic conditions that resemble the microenvironment of inflamed skin wounds.

Additionally, (Agrawal et al., 2023) highlighted he potent antimicrobial effects of *Ziziphus jujuba* fruit extract against common wound pathogens, suggesting that reduced infection rates in treated wounds may be linked to the extract's bioactive polyphenolic profile. Based on these studies, the wound-healing activity of *Ziziphus jujuba* appears to be mediated through a combination of antimicrobial suppression, antioxidative protection, modulation of inflammatory mediators, and stimulation of extracellular matrix formation.

Variations in healing magnitude between these studies and the present research may stem from differences in plant parts used (fruit vs. leaf), extraction solvents, phytochemical concentrations, wound models, or dosing regimens.

However, despite these methodological differences, the overall trend across the literature consistently supports that increased concentrations of *Ziziphus jujuba* extract enhance wound healing efficiency through mechanisms that include suppression of pathogenic bacteria and reduction of inflammatory responses.

CONCLUSION

From this study, it can be concluded that the administration of *Ziziphus jujuba* extract ointment has the potential to accelerate the healing of burns macroscopically, where the group with the highest concentration (20 mg/mL) showed a higher rate of reduction in burn diameter, minimal local and allergic reactions, and contributed to a shorter wound healing time compared to other groups. Although the differences between groups have not been statistically significant, consistent pattern of results indicates a biologically meaningful effect. This trend aligns with the known pharmacological properties of *Ziziphus jujuba*, particularly its anti-inflammatory effects which may contribute to improved wound contraction and shorter healing duration.

Overall, this study indicate that *Ziziphus jujuba* extract exhibits promising therapeutic properties for incorporation into burn-healing ointment formulations, suggesting its potential as an alternative therapeutic option for burn wound management. To substantiate these preliminary findings, future research should include broader dosage optimization, longer study durations with larger sample populations, and well-designed clinical evaluations in humans to verify its safety profile and therapeutic efficacy in clinical practice.

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