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Efficacy of *Biophytum petersianum* Klotzsch Extract Ointment on the Healing of Burns in Rats (*Rattus norvegicus*)

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ABSTRACT

Background: *Biophytum petersianum* Klotzsch has traditional uses in wound treatment and reported immunomodulatory and antioxidant activities, but evidence of its topical application for burn healing remains limited.

Objective: This study evaluated the effectiveness of *B. petersianum* Klotzsch extract ointment at 5%, 7.5%, and 10% on burn healing in rats (*Rattus norvegicus*).

Methods: Rats were divided into five groups: positive control (Bioplacenton), negative control (ointment base), and three treatment groups receiving *B. petersianum* Klotzsch extract ointment at 5%, 7.5%, and 10%. Wound healing was assessed on days 1, 7, and 14 based on the percentage of wound closure. Data were analyzed using one-way ANOVA followed by LSD at $\alpha = 0.05$.

Results: No significant differences were observed among groups on day 1 ($p > 0.05$), whereas significant differences occurred on days 7 and 14 ($p < 0.05$). Baseline analysis showed significant differences among groups ($p = 0.025$), indicating incomplete initial wound homogeneity. LSD analysis demonstrated increasingly evident differences at later observation times, particularly for the 7.5% and 10% formulations compared with the negative control on day 14. The 10% formulation showed the highest overall healing response, although the dose–response pattern was not consistently linear.

Conclusion: *B. petersianum* Klotzsch extract ointment showed potential to improve burn healing in rats, particularly during later healing stages. However, baseline differences and the absence of mechanistic and histopathological assessments limit definitive interpretation. Further studies are needed to establish the optimal concentration, safety, formulation stability, and mechanism of action.

Keywords: *Biophytum petersianum*; Herbal Ointment; Wound Healing; Rats; Topical Formulation

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INTRODUCTION

Burns are a complex form of tissue damage because they not only cause loss of skin integrity, but also trigger inflammatory responses, oxidative stress, cell damage, and an increased risk of infection (Mulder et al., 2022; Prabawati et al., 2021; Żwierełło et al., 2023). The wound healing process takes place through interrelated phases of hemostasis, inflammation, proliferation, and remodeling. An imbalance in one of these phases can slow the closure of the wound and increase the risk of complications (Fernández-Guarino et al., 2023; Greenhalgh & Kiley, 2024; Patenall et al., 2024; Yan et al., 2024). Therefore, burn therapy is not enough to prevent infection, but also needs to support tissue regeneration and control inflammation and oxidative stress. In recent years, attention to natural materials as burn therapy candidates has been increasing. Recent systematic reviews show that various medicinal plants can accelerate the healing of burns through antioxidant, anti-inflammatory, antibacterial, cell proliferation stimulation, angiogenesis, and collagen formation (Bagheri et al., 2025; Souza-Silva et al., 2026). Topical formulations, specifically ointments, are also one of the most widely used forms of preparations in preclinical research because they allow the active ingredient to come into direct contact with wound tissue. Clinical and preclinical evidence also suggests that some natural ingredients, such as *Aloe vera* and honey, have the potential to improve burn healing time compared to certain standard therapies, although their effectiveness is highly dependent on the type of plant, formulation, concentration, and wound characteristics (Huang et al., 2024; Nasiri et al., 2015; Roşca, Nistor, et al., 2025).

One of the interesting local plants to develop is *Biophytum petersianum* Klotzsch, which is known as kebar grass and is a medicinal plant native to Papua. Previous research has shown that *B. petersianum* Klotzsch extract contains a variety of secondary metabolites, especially flavonoids, tannins, saponins, and phenolic compounds (Aminudin et al., 2020; Lisangan et al., 2023; Suryani et al., 2026). The extract also shows quite strong antioxidant activity, with a relatively high total phenolic content. More recent findings also show that water and ethanol extracts *of B. petersianum* Klotzsch have activity in inhibiting nitric oxide (NO), thus providing a biological basis that these plants have the potential to modulate inflammatory processes and nitrosative stress (Aminudin et al., 2020; Lisangan et al., 2023; Suryani et al., 2026).

Ethnopharmacologically, *B. petersianum* Klotzsch also has a history of traditional use as a plant to aid in wound healing. Phytochemical studies of these plants have even identified polysaccharide fractions with biological activity associated with the complement system, providing clues that plant components may interact with immunological mechanisms relevant to the healing process (K. T. Inngjerdingen et al., 2005; M. Inngjerdingen et al., 2008; Malterud, 2017). However, the evidence cannot yet be considered evidence of clinical or therapeutic efficacy against burns. Most *B. petersianum* Klotzsch research still focuses on antioxidant activity, secondary metabolite content, and other reproductive and pharmacological effects, while experimental evidence specifically evaluating *B. petersianum* Klotzsch extract in ointment form in burn models is still very limited (Aminudin et al., 2020; K. T. Inngjerdingen et al., 2005; M. Inngjerdingen et al., 2008). The gap is important because the biological activity of an extract does not automatically guarantee its effectiveness when formulated as a topical preparation. The concentration of the extract, the base of the ointment, the release and penetration ability of the active compound, as well as the length of contact with the tissue may affect the healing response. Studies on other medicinal plants suggest that topical formulation of extracts can produce a noticeable difference in effects on wound contraction and tissue regeneration, including in mouse burn models. Thus, experimental testing was needed that directly assessed the effectiveness of *B. petersianum* Klotzsch extract ointment in controlled burn models (Roşca, Coneac, et al., 2025; Thanapaul et al., 2020).

This research is important because it links the pharmacological potential of local Papuan plants with the development of topical preparation candidates for burn healing. If proven effective, *B. petersianum* Klotzsch not only has value as a source of bioactive compounds, but also has the potential to be developed into a raw material for phytopharmaceuticals or topical preparations based on local plants. This approach is in line with the need to find natural ingredient-based therapies that are effective, affordable, and have a stronger preclinical evidence base. However, the interpretation of its therapeutic benefits still needs to be based on measurable healing parameters and compared to the appropriate control group, rather than solely based on phytochemical content or antioxidant activity. Therefore, this study aims to evaluate the efficacy of *B. petersianum* Klotzsch extract ointment in accelerating the healing of burns in white rats (*Rattus norvegicus*). The research questions posed were: did administration of *B. petersianum* Klotzsch extract ointment improve the healing process of burns in mice compared to controls, and did the effects differ based on extract concentrations? The research hypothesis is that the ointment extract of B*. petersianum* Klotzsch. It has a better burn healing effect compared to the control, which is demonstrated by increased wound contraction and/or accelerated wound closure.

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METHODS

Research Design and Setup

This study is a laboratory experimental research with a true experimental post-test only control group design that aims to evaluate the effectiveness of *B. petersianum* Klotzsch extract ointment on the healing of burns in white rats (*Rattus norvegicus*). The experimental animals were randomly divided into five groups, namely negative control (K−), positive control (K+), and three treatment groups that received *B. petersianum* Klotzsch extract ointment with concentrations of 5%, 7.5%, and 10%. Group K− received an ointment base without extract, while group K+ received a Bioplacenton preparation as a therapeutic comparator. The treatment group received *B. petersianum Klotzsch extract ointment* with concentrations of 5% (FI), 7.5% (FII), and 10% (FIII), respectively. All groups were treated and observed under the same environmental conditions.

Experimental Animals and Data Sources

The source of research data is primary data obtained through direct observation of the burn healing process in rats (*Rattus norvegicus*). The experimental animals used were healthy, active, not physically abnormal, and met the criteria set in the research protocol. Before treatment, the mice underwent an acclimatization period to adjust to the conditions of the laboratory environment. During the study, animals were raised with access to adequate feed and drinking water as well as relatively uniform environmental conditions.

Sample Collection and Allocation

Experimental animals that met the inclusion criteria were allocated into five study groups using a randomization method to reduce the likelihood of selection bias. Each rat was assigned an identification code and placed in a predetermined treatment group. The number of mice in each group should be explicitly reported in the manuscript, including the basis for determining the number of samples, as such information is needed to ensure the reproducibility of the study.

This study used mice test animals that were divided into several groups, with each group consisting of 3 mice (n = 3). The use of the number of test animals considers the *3R (Replacement, Reduction, and Refinement)* principle in animal research ethics, especially the *Reduction aspect*, which is to minimize the use of test animals.

Ointment Formulation

Biophytum petersianum Klotzsch extract is formulated into an ointment preparation with three concentrations, namely 5%, 7.5%, and 10%. Based on the research formulation, each treatment preparation is made with a total weight of 25 g. The composition of each formula is as follows:

BahanIngredients	Control -	Control +	Formula Dose 5%	Formula Dose 7.5%	Formula Dose 10%
Bioplacenton	–	15 g	–	–	–
Extract *B. petersianum* Klotzsch	–	–	1.25 g	1.875 g	2.5 g
Liquid paraffin	8.5 g	–	8.5 g	8.5 g	8.5 g
Yellow petroleum	16.5 g	–	15.25 g	14.625 g	14 g
Total	**25 g**	**15 g**	**25 g**	**25 g**	**25 g**

In group K−, the ointment base consists of 8.5 g of liquid paraffin and 16.5 g of yellow petroleum with a total weight of 25 g. The FI formula contains 1.25 g of extract, 8.5 g of liquid paraffin, and 15.25 g of yellow vaseline. The FII formula contains 1,875 g of extract, 8.5 g of liquid paraffin, and 14,625 g of yellow vaseline, while the FIII contains 2.5 g of extract, 8.5 g of liquid paraffin, and 14 g of yellow vaseline. The K+ group used 15 g of Bioplacenton as stated in the research formulation. To ensure reproducibility, the ointment manufacturing process is carried out by weighing all ingredients using a calibrated scale, then mixing the ingredients homogeneously until a preparation with a uniform consistency is obtained. Storage conditions of preparations, such as containers, temperature, and storage time before use, need to be included in the research protocol.

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Burn Induction

Burns are made on the skin area of the rat that has been determined using a standardized burn induction method. Before induction, the skin area is shaved and cleaned, then the animal is given anesthesia according to the ethical protocol of the research. Induction is carried out using the same heat source throughout the animal so that the size and degree of the wound can be made as homogeneous as possible. After the burns formed, the rats were given treatment according to their group. Group K− was given an ointment base, group K+ was given Bioplacenton, while groups FI, FII, and FIII were given ointment of *B. petersianum* Klotzsch extract with concentrations of 5%, 7.5%, and 10%, respectively. The preparation was applied topically to the wound area with the same amount, frequency, and time of administration for the rest of the group. To support reproducibility, the final research method must specifically include the temperature of the heat source, the diameter of the appliance or the area of the contact surface, the length of contact, the degree of burn, the dose of anesthesia, the amount of ointment applied, the frequency of application, and the duration of therapy.

Wound Healing Measurement

The effectiveness of wound healing was assessed based on changes in wound size during the observation period. The extent of the wound was measured using the same measurement method in all groups and was carried out at a predetermined observation time.

Measurement Validity and Reliability

Internal validity is maintained by using test animals that meet the same criteria, uniform burn induction procedures, the same maintenance conditions, and consistent preparation methods. The concentration of each preparation is made based on a predetermined formula, and all ingredients are weighed using the appropriate measuring instrument. Measurement reliability is improved by using the same instrument across observations, taking measurements at a consistent time, and using uniform wound documentation procedures. When measurements are taken photographically, camera distance, lighting, animal position, and measurement scale must be consistent. Measurements should preferably be taken by the same observer or, if possible, by an observer who is not aware of the treatment group to reduce *observer bias*.

Data Collection

Data were collected prospectively during the study period. The data recorded included animal codes, treatment groups, weight, initial wound area, wound area at each observation time, and wound healing percentage calculation results. All observations are recorded on the research observation sheet and then entered into an electronic database. The data is double-checked before analysis to ensure completeness and consistency of the recordings.

Data Analysis

Quantitative data is presented as an average ± standard deviation (mean ± SD). Before parametric analysis is carried out, the data is tested to ensure the fulfillment of the analysis assumptions, namely the normality and homogeneity of variance tests. The normality test can be performed using Shapiro–Wilk, while the homogeneity of variance is tested using Levene's test**.** The difference in wound healing effectiveness between the five groups was analyzed using a one-way analysis of variance (one-way ANOVA). If the ANOVA results show significant differences between groups, the analysis is continued with the Least Significant Difference (LSD) test to determine the group pairs that have significant differences. The level of statistical significance is set at p-value = 0.05. A p-value of < 0.05 is considered to indicate a statistically significant difference.

Software

Data processing and analysis are carried out using IBM SPSS Statistics. Data is entered into the software after checking the completeness and consistency of the data. The analysis included descriptive statistics, normality tests, homogeneity tests, one-way ANOVA, and LSD follow-up tests.

Research Ethics

This study uses experimental animals, so the entire procedure is carried out by paying attention to the principles of animal welfare and the 3Rs (Replacement, Reduction, and Refinement) principle**.** The animals were kept in appropriate conditions, received adequate feed and water, and were monitored during the study

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period. Procedures that have the potential to cause pain, including burn induction, are performed using anesthesia and pain management measures according to approved protocols. The animal's condition was monitored periodically during the study to identify signs of pain, infection, or worsening clinical conditions.
This research has received ethical approval from the Health Research Ethics Commission of the Makassar College of Pharmaceutical Sciences with ethics certificate number 332/EC.1.1/VII/KEPK/2024. All research procedures are carried out in accordance with the protocol that has received ethical approval.

Reproducibility of the Research

To allow the research to be replicated, the research method must fully list the identity and characteristics of the test animal, the number of mice in each group, the method of randomization, the source and authentication of plant ingredients, the method of extraction and yield of the extract, the procedure for making the ointment, the storage conditions of the preparation, the method of induction of the burn and its technical parameters, the number and frequency of application of the ointment, the length of observation, the method of measuring the area of the wound, as well as the statistical procedures used. The formulation information used in the study has been quantitatively determined, so that FI, FII, and FIII formulas can be recreated by 25 g each with an extract composition of 5%, 7.5%, and 10%. The use of negative controls in the form of ointment bases and positive controls in the form of bioplacenton allowed evaluation of the pharmacological effects of extracts compared to conditions without active ingredients and comparative therapies.

Results

The results showed a change in the percentage of burn healing in all treatment groups during the observation period. Measurements were taken on the 1st, 7th, and 14th days to evaluate the progress of wound healing after the administration of *B. petersianum* Klotzsch extract ointment at various concentrations. In general, the percentage of cure increases as the observation time increases, with different responses between treatment groups.
Statistical analysis was carried out using One-Way Analysis of Variance (ANOVA) to determine the difference in the percentage of wound healing between groups at each observation time. If there is a significant difference, the analysis is continued using the Least Significant Difference (LSD) to identify group pairs that show significant differences. The results of the descriptive and statistical analysis of the percentage of burn healing are presented in Table 1, while the results of the comparison between groups based on the LSD test are presented in Table 2.
In initial observations, the condition of the lesions did not show consistent differences between groups. Over time, the percentage of cure increased, especially in the group that obtained the *B. petersianum* Klotzsch extract ointment. The differences between groups became more pronounced on day 7 and remained more pronounced on day 14. These findings suggest a progression of wound healing processes during the observation period, although the magnitude of the response differed based on treatment group and extract concentration.

Table 1. Data on the average percentage of burn healing (Δ%) in each intervention group

Variable	Control +	Control -	Dose 5%	Dose 7.5%	Dose 10%	p-value
Pret-test	2.45±0.14	2.55±0.06	2.39±0.06	2.31±0.11	2.23±0.11	0.025*
			Post-test (Δ%)			
Day 1	3.44±2.24	0.65±0.60	5.90±4.77	6.88±3.66	3.03±2.68	0.191
Day 7	39.09±2.90	7.79±9.83	9.52±4.68	22.56±11.35	33.26±15.12	0.010*
Day 14	100±0.00	26.23±11.46	57.85±36.62	82.81±29.77	100±0.00	0.008*

Note: Data are presented as **mean ± SD** (*mean ± standard deviation*). **SD** = *standard deviation* (standard deviation); **Δ%** = percentage of wound change/healing. **Control (+)** = positive control group and **Control (−)** = negative control group. The differences between groups were analyzed using **One-Way Analysis of Variance (ANOVA)** with a significance level of $p < 0.05$. The sign (*) indicates a statistically significant difference.

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The results of the analysis using One-Way ANOVA showed that there was a statistically significant difference in the percentage of burn healing between groups on day 7 and day 14 ($p < 0.05$). On the other hand, on day 1 there was no significant difference between groups ($p > 0.05$), which suggests that the effect of treatment on wound healing has not been noticeable in the early phase. The results of the pre-test analysis showed that there was a significant difference between groups ($p = 0.025$), so that the initial condition of the wound between groups was not completely homogeneous. On days 7 and 14, the group given *B. petersianum* Klotzsch extract ointment showed a greater percentage increase in healing as concentrations increased, with a concentration of 10% indicating the most optimal healing response.

Table 2. Comparison of the efficacy of using *Biophytum petersianum* Klotzsch extract ointment for the healing of burns in rats (*Rattus norvegicus*)

Measurement Time	Groups		p-value	95% CI (Lower±Upper)
Day 1	Control +	Dose 5%	0.358	-8.13±3.22
		Dose 7.5%	0.207	-9.11±2.24
		Dose 10%	0.875	-5.27±6.09
	Control -	Dose 5%	0.067	-10.92±0.43
		Dose 7.5%	0.035*	-11.90±-0.55
		Dose 10%	0.372	-8.06±3.30
Day 7	Control +	Dose 5%	0.004*	11.66±47.47
		Dose 7.5%	0.065	-1.27±34.53
		Dose 10%	0.485	-12.07±23.73
	Control -	Dose 5%	0.834	-19.63±16.17
		Dose 7.5%	0.098	-32.57±3.24
		Dose 10%	0.010*	-43.37±-7.56
Day 14	Control +	Dose 5%	0.039*	2.64±81.67
		Dose 7.5%	0.355	-22.32±56.70
		Dose 10%	1.00	-39.51±39.51
	Control -	Dose 5%	0.105	-71.12±7.90
		Dose 7.5%	0.010*	-96.09±-17.06
		Dose 10%	0.002*	-113.28±-34.25

Note: LSD = *Least Significant Difference*, which is *a post hoc* test to find out significantly different pairs of groups after ANOVA analysis shows differences between groups. **CI** = *Confidence Interval* (95% confidence interval). **Control (+)** = positive control and **Control (−)** = negative control. Comparison data are presented with **a 95% CI.** The difference is considered significant if $p < 0.05$ and is denoted by (*).

Table 2 shows the results of the analysis of the Least Significant Difference (LSD) follow-up test to compare the effectiveness of kebar grass extract ointment with the control group at each observation time. On day 1, most comparisons did not show a significant difference, although there was a significant difference between the negative control and the 7.5% dose ($p < 0.05$). On day 7, significant differences were mainly seen between the positive control and the 5% dose, as well as between the negative control and the 10% dose. On day 14, a significant difference was found between the positive control and the 5% dose, as well as between the negative control and the 7.5% and 10%

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doses. These findings suggest that wound healing responses tend to differ increasingly between groups over longer observation periods, especially at higher concentrations of extracts.

DISCUSSION

The results of ANOVA analysis at the pre-test stage showed a significant difference in the average initial condition of the wound between the treatment groups ($p = 0.025$). These findings indicate that the condition of the wound before treatment was not completely equal. These differences in initial conditions have the potential to affect the wound healing response and are a factor that needs to be considered in interpreting the effectiveness of treatment. Groups with different initial wound conditions may show different patterns and healing rates, so the difference in final outcome cannot be fully attributed to the effect of the treatment given.

The results of the Least Significant Difference (LSD) follow-up test showed that the effectiveness of *B. petersianum* Klotzsch extract ointment on burn healing was time-dependent, but did not show a completely linear pattern of dose-response relationships. On day 1, most of the groups had not shown any significant difference compared to the controls. This is biologically understandable because the early phases of wound healing are still dominated by hemostasis and inflammatory responses, so the topical effect on tissue regeneration processes is not yet fully visible.

On day 7, a more pronounced difference began to be seen. The 5% dose showed a significant difference compared to the positive control, while the 10% dose showed a significant difference compared to the negative control. These findings indicate that the administration of the extract began to contribute to the healing process in the proliferative phase. However, because not all doses differed significantly from the two controls, the results were not enough to conclude that higher concentrations of the extract always resulted in better healing effects. On the 14th day, the differences between the groups became more pronounced. Doses of 7.5% and 10% showed significant differences compared to negative controls, while doses of 5% also showed significant differences in positive controls. Thus, the results of the study are more accurately interpreted as indicating that *B. petersianum* Klotzsch extract ointment has the potential to accelerate the healing of burns, especially in the advanced healing period, but the concentration that provides the most optimal effect needs to be determined based on the overall analysis, not just based on the p-value of the comparison of group pairs. The wound healing response to the administration *of B. petersianum* Klotzsch extract concentrations of 7.5% and 10% on day 7 showed a non-linear pattern of increased concentration. This condition is thought to be related to variations in individual biological responses of mice, differences in initial wound conditions, and dynamics of inflammatory and proliferation phases in the healing process. In addition, increased extract concentrations do not necessarily result in a proportional increase in effectiveness due to the possibility of optimal biological response limits and differences in penetration of active compounds in wound tissue. A relatively small number of test animals, i.e. three mice in each group ($n = 3$), may also increase the influence of individual variation on observational outcomes.

Biologically, the findings have a fairly plausible basis. *B. petersianum* Klotzsch has been reported to have traditional uses as a plant to aid in wound healing. Studies of pectic polysaccharides from the aerial part *of B. petersianum* Klotzsch also showed immunomodulatory activity, including activity on the complement system as well as macrophages and dendritic cells. The activity is interesting because the regulation of the inflammatory response is an important part of the transition to the proliferation and remodeling phases in wound healing. However, the activity of such immunomodulators cannot be directly considered evidence that the ointment extract in this study works through the same mechanism, as the current study does not measure inflammatory mediators, protein expression, or histological parameters (Arfiani et al., 2023; K. T. Inngjerdingen et al., 2005; M. Inngjerdingen et al., 2008; Mulder et al., 2022).

Previous evidence also supports the potential of *B. petersianum* antioxidants. The extract reportedly contains flavonoids, tannins, and saponins and exhibits antioxidant activity, while more recent research has also reported nitric oxide-inhibiting activity from *Klotzsch's* B. petersianum extract. Theoretically, antioxidant activity may be relevant in wound healing because excessive oxidative stress can interfere with the tissue repair process. However, because this study did not measure levels of ROS, MDA, SOD, or other oxidative biomarkers, the antioxidant mechanism should not be stated as a mechanism that has been proven in this study.

The results of this study are also conceptually in line with the broader literature on the use of medicinal plants in burns. A recent systematic review of 108 preclinical and clinical studies reported that various medicinal plants have the potential to aid in burn healing through antioxidant, anti-inflammatory, antibacterial, and modulating the various phases of wound healing. Similarly, a meta-analysis of *Aloe vera* in second-degree burns

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found a faster healing time compared to comparative therapy, although the findings were derived from different plants and formulations, so they could not be directly extrapolated to *B. petersianum* Klotzsch (Lisangan et al., 2023; Mulder et al., 2022; Suryani et al., 2026).

The effectiveness of *Biophytum petersianum* Klotzsch extract in supporting the wound healing process is thought to be related to the potential immunomodulatory and antioxidant activity of the bioactive compounds it contains. Theoretically, these activities may contribute to the regulation of inflammatory responses and tissue protection against oxidative stress during the wound healing process. However, this study did not measure biomarkers of oxidative stress, such as *reactive oxygen species* (ROS) and *malondialdehyde* (MDA), nor did it perform histopathological examination of wound tissue to evaluate cellular changes and tissue regeneration processes. Therefore, the involvement of immunomodulatory and antioxidant mechanisms in the observed wound healing effects is still hypothetical and cannot be directly confirmed. The findings of this study mainly show changes in wound healing based on the observed parameters, without proving an underlying molecular or cellular mechanism. Further research is needed to evaluate inflammatory and oxidative stress biomarkers as well as tissue histopathological characteristics to clarify the biological mechanisms that may be involved.

From a theoretical perspective, this study expands the preclinical evidence regarding the utilization *of B. petersianum* Klotzsch from antioxidant aspects and traditional use to its possible application as a topical preparation for burn healing (Aminudin et al., 2020; K. T. Inngjerdingen et al., 2005; Malterud, 2017; Suryani et al., 2026). The important value of the research lies not only in the existence of statistical differences, but in the indication that the formulation of the extract in the form of an ointment has the potential to provide a local effect during the healing process. However, more research is needed to determine the relationship between extract concentration, active compound content, and tissue biological response. From a managerial perspective and the development of pharmaceutical preparations, these results provide an initial basis for considering *B. petersianum* Klotzsch as an active ingredient candidate in the development of topical preparations based on natural ingredients. However, the results in the mouse model cannot be used as a basis for recommending clinical use in burn patients. Further development should include standardization of extracts, characterization of marker compounds, evaluation of the stability and physical quality of preparations, safety/skin irritation, histopathological examinations, and studies with a more robust design before being translated into clinical research. The limited evidence on a wide range of topical, herbal-based therapies also demonstrates the importance of controlled research design and good reporting before clinical effectiveness can be ascertained.

CONCLUSION AND IMPLICATIONS

B. petersianum Klotzsch extract ointment showed potential in improving burn healing in rats (*Rattus norvegicus*), especially at the observation of the 7th and 14th days. The healing effect showed variation between concentrations, so an increase in dose cannot be directly considered to result in a better response. Theoretically, these findings strengthen the preclinical evidence regarding the potential *of B. petersianum* Klotzsch extract ointment as a natural ingredient for topical wound healing preparations. Managerially, the results of the research can be the initial basis for the development and standardization of *B. petersianum* Klotzsch extract -based ointment preparations, but they are not yet sufficient to support clinical use. Further research is needed to evaluate the mechanism of action, safety, stability of the preparation, histopathological parameters, and clinical effectiveness before it can be recommended as burn therapy.

Limitations and Future Research

This study has several limitations that need to be considered in interpreting the findings. First, the study used a model of the animal *Rattus norvegicus*, so the results could not be directly generalized to humans. Second, the evaluation parameters are mainly based on changes in the percentage of wound healing macroscopically, so the biological mechanism underlying the effect of *B. petersianum* Klotzsch extract ointment cannot be ascertained. Third, studies have not evaluated histopathological parameters, inflammatory biomarkers, oxidative stress, and growth factor expression that could provide stronger evidence regarding healing mechanisms. In addition, the study has not included a comprehensive evaluation of the safety, skin irritation, stability, and standardization of the content of active compounds in ointment preparations.

Further research needs to focus on the standardization of extracts and formulations, the identification and quantification of active compounds, and the determination of optimal concentrations through the design of stronger dose-response relationships. Subsequent studies are also suggested to integrate histopathological

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examinations and biomarkers of inflammation and oxidative stress to elucidate the mechanism of action more specifically. Evaluation of the toxicity and safety of topical use needs to be done before translation into clinical research. Once such preclinical evidence is adequate, controlled clinical studies may be considered to determine the effectiveness and safety of *B. petersianum* Klotzsch extract ointment in patients with burns.

DECLARATIONS

Ethics approval: This study was conducted in accordance with applicable ethical principles for the care and use of laboratory animals. Ethical approval was obtained from the Health Research Ethics Committee of Sekolah Tinggi Ilmu Farmasi Makassar, Indonesia, under Ethics Approval No. 332/EC.1.1/VII/KEPK/2024. All experimental procedures involving *Rattus norvegicus* were performed in accordance with the approved ethical protocol, with appropriate measures taken to minimize animal discomfort, pain, and distress throughout the study.

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Author contributions: All authors contributed to the conception and design of the study, data collection, data analysis, interpretation of the results, and preparation and revision of the manuscript. All authors have read and approved the final version of the manuscript.

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