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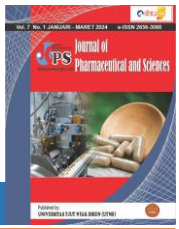

# Efficacy of *Nothopanax sculleturium* (mangkokan leaf) and *Colocasia esculenta* L. (taro stalk) ointment in the healing of male rabbit incised wounds.

# Uji efektivitas salep kombinasis *Nothopnax sculleturium* (daun mangkokan) dan *Colocasia esculenta* L. (daun talas) terhadap penyembuhan luka sayat kelinci jantan

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**Abstract**

**Background:** *Nothopanax scutellarium* (mangkokan leaves) and *Colocasia esculenta L.* (taro petioles) contain bioactive compounds such as flavonoids, saponins, tannins, terpenoids, and steroids, which play essential roles in wound healing. **Objective:** This study aimed to evaluate the effectiveness of a topical ointment containing a combination of ethanolic extracts of mangkokan leaves and taro petioles on incised wound healing in male rabbits. **Methods:** A laboratory experimental study with a *post-test-only control group design was conducted using three ointment formulas (10%:5%, 7.5%:7.5%, and 5%:10%), alongside positive (povidone-iodine*) and negative controls. Wound length was measured daily for 10 days. Data were analysed using one-way ANOVA followed by Tukey's post-hoc test at a significance level of $\alpha = 0.05$. **Results:** All formulations significantly accelerated wound healing compared with the negative control ($p < 0.05$). The 5%:10% formulation achieved complete wound closure on day 7, showing a statistically significant difference from the positive control ($p < 0.05$). **Conclusion:** The combination of mangkokan leaf and taro petiole extracts effectively promotes incised wound healing, with the 5%:10% formula demonstrating the most optimal effect. These findings support the potential of developing this herbal ointment as a safe and efficient antiseptic alternative.

***Keywords: Mangkokan leaf, Taro stalk, incised wound, antiseptic ointment, rabbit model***

**Abstrak**

**Latar Belakang**: Daun mangkokan (Nothopanax scutellarium) dan tangkai daun talas (Colocasia esculenta L.) mengandung flavonoid, saponin, tanin, terpenoid, dan steroid yang berperan dalam proses penyembuhan luka. **Tujuan:** Mengevaluasi efektivitas kombinasi ekstrak etanol daun mangkokan dan tangkai daun talas dalam sediaan salep terhadap penyembuhan luka sayat pada kelinci jantan. **Metode**: Penelitian eksperimental post-test only control group design ini menggunakan tiga formula salep (10%:5%, 7,5%:7,5%, dan 5%:10%) dengan kontrol positif (povidone iodine) dan kontrol negatif. Panjang luka diukur setiap hari selama 10 hari. Data dianalisis menggunakan ANOVA satu arah dan uji lanjut Tukey HSD ($\alpha = 0,05$). **Hasil**: Semua formula mempercepat penyembuhan luka secara signifikan dibandingkan dengan kontrol negatif ($p < 0,05$). Formula 5%:10% menunjukkan penyembuhan sempurna pada hari ke-7 dan berbeda secara signifikan dibandingkan dengan kontrol positif ($p < 0,05$). **Kesimpulan**: Kombinasi ekstrak daun mangkokan dan tangkai daun talas efektif dalam mempercepat penyembuhan luka sayat, dengan rasio 5%:10% sebagai konsentrasi yang paling optimal. Temuan ini mendukung potensi pengembangan salep herbal sebagai alternatif antiseptik yang aman dan efektif.

***Kata Kunci: Daun mangkokan, tangkai daun talas, luka sayat, salep antiseptik, model kelinci.***



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## Introduction

Skin is the largest organ in the human body and functions as the first physical barrier against pathogen invasion and microbial colonisation. In addition, the skin also contributes to various other defence mechanisms of the body. Damage to the integrity of the skin, mucosa, or other organs is referred to as a wound, which may result in several consequences such as loss of organ function, bleeding, bacterial contamination, and cell death. [1]. The wound healing process consists of three major phases: the inflammatory phase (day 0 to day 7), the regeneration phase (day 3 to day 4), and the remodelling phase (3–12 months or longer).

Wounds can be classified into several types, including abrasions (*Vulnus excoriasi*), incised wounds (*Vulnus scissum*), lacerations (*Vulnus laseratum*), puncture wounds (*Vulnus punctum*), bite wounds (*Vulnus morsum*), and burn wounds (*Vulnus combustionis*). Incised wounds are characterised by linear tissue separation generally caused by sharp objects and may also be intentionally created for medical purposes. [2]. Appropriate wound management is essential to prevent infection. Povidone-iodine is a widely used antiseptic; however, its use is increasingly being reconsidered because of its potential to damage healthy cells and tissues. [3]. Therefore, the use of natural materials as safer and more cost-effective therapeutic alternatives has gained growing attention.

One plant that shows promising potential is mangkokan leaf (*Nothopanax scutellarium*), which is known to contain flavonoids, alkaloids, saponins, and polyphenols. [4]. Traditionally, these leaves have been utilised to accelerate wound healing and are reported to possess antibacterial, anti-inflammatory, and antioxidant activities. Previous research has indicated that mangkokan ointment extract at concentrations of 50–75% is effective in the treatment of burn wounds. [5]. Another plant with potential is taro (*Colocasia esculenta* L.), particularly the leaf stalk, which is rich in flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids. These compounds act as antibacterial, anti-inflammatory, and astringent agents. A cream formulation containing ethanol extract of taro stalk is effective in healing incised wounds in rats, with an optimal concentration of 15% [6,7]. In addition, a gel containing 5% dried taro extract demonstrated high effectiveness in healing burn wounds within 13 days .

Previous studies on mangkokan leaves and taro leaf stalks have generally been conducted separately, using single preparations such as ointments, creams, or gels, and have mostly focused on burn or incised wounds in rats. These studies also employed only one extract concentration, so the effectiveness of combining both extracts in a single ointment formulation remains unknown. Furthermore, no direct comparison with povidone-iodine has been performed, and experimental data using rabbits are still very limited. Based on this background, the present study aims to evaluate the effectiveness of a combination of ethanol extracts of mangkokan leaves and taro leaf stalks formulated as an ointment in healing incised wounds in male rabbits, as well as to determine the optimal combination concentration.

## Methods

This study was a quantitative experimental laboratory investigation aimed at evaluating the effectiveness of an antiseptic ointment formulated from a combination of ethanol extracts of mangkokan leaves (*Nothopanax scutellarium*) and taro leaf stalks (*Colocasia esculenta* L.) in promoting the healing of incised wounds in male rabbits. The research was conducted from June to August 2023 at the Pharmacognosy, Semi-Solid, and Pharmacology Laboratories of the Helvetia Institute of Health. Ethical approval for the study was obtained from the Faculty of Nursing, Universitas Sumatera Utara (No. 2875/VIII/SP/2023).

The study applied a post-test-only control group design with repeated measurements. Uniform incised wounds measuring 1.2 cm were created on all experimental animals. Each group then received a different treatment: a negative control, a positive control, and three formulations containing combinations of plant extracts. Wound length was measured daily until complete healing occurred. Randomisation was performed using simple random allocation, in which all rabbits were assigned identification numbers and randomly distributed into five treatment groups using a random number table. Each group consisted of three rabbits. The sample size was determined by considering the Reduction principle of animal ethics, the homogeneity of experimental animals, and consistency with previous preliminary animal studies. This minimum sample size limits statistical power and the generalizability of the findings.

**Extract Preparation**



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The extracts were prepared using the maceration method. A total of 5 kg of fresh plant material was dried at 50°C and then ground into powder. Approximately 500 g of the powdered material was macerated with 70% ethanol at a ratio of 1:10 (3750 mL). The mixture was stored in a closed container protected from light for five days and stirred occasionally. The first filtrate was collected by filtration, and the remaining residue was re-macerated with 1250 mL of ethanol for three days. This process was repeated twice. All filtrates were subsequently combined and concentrated using a rotary evaporator to obtain a thick extract. [5]. The extracts were then formulated into three ointment preparations with different concentration combinations: F1 (10% mangkokan: 5% taro), F2 (7.5%: 7.5%), and F3 (5%: 10%). The control groups consisted of a positive control (povidone-iodine ointment) and a negative control (ointment base without active ingredients). Vaseline flavum was used as the ointment base because it provides excellent occlusive properties, good stability, and prolonged retention of active compounds on the wound surface.

**Testing Procedure and Data Analysis**

The experiment involved 15 healthy male rabbits, which were randomly assigned to five treatment groups. A 1.2 cm incised wound was created aseptically on the dorsal area of each rabbit, and the ointment was applied once daily for 10 days. Wound length was measured daily using a calliper to evaluate the wound healing process quantitatively.

**Table 1.** Ointment Preparation Formula and Composition

| Ingredient | F0 | F1 | F2 | F3 |
|---|---|---|---|---|
| Mangkokan Extract | 0 | 10 | 7.5 | 5 |
| Taro Extract | 0 | 5 | 7.5 | 10 |
| Vaselinum flavum | 100 | 85 | 85 | 85 |

In addition, the physical quality of the ointment formulations was assessed through organoleptic evaluation, homogeneity testing, spreadability testing, and pH measurement to ensure that the topical preparations met pharmaceutical standards. [8]. Statistical analysis was performed using Analysis of Variance (ANOVA) to determine differences in wound healing effectiveness among treatment groups on each observation day. The test was conducted at a significance level of $\alpha = 0.05$, and all statistical analyses were carried out using IBM SPSS Statistics version 25. To identify which groups showed significant differences, a Tukey HSD post-hoc test was performed with the same significance level ($\alpha = 0.05$).

## Results and Discussion

**Organoleptic Evaluation of Ointment Formulations**

The organoleptic evaluation was conducted by observing the ointment preparations based on their physical form, colour, and odour over three observation cycles, from day 1 to day 9. The results showed that the ointment formulations containing the combination of mangkokan leaf extract and taro leaf stalk extract produced two formulations with a darker green colour and one formulation with a light yellow colour, all with a semi-solid consistency and a distinctive extract aroma. However, differences were observed among the formulations due to variations in the concentration of the combined extracts of mangkokan leaves and taro leaf stalks. These differences influenced the appearance and uniformity of the ointments. The most intense colour was observed in Formula 1 (10%:5%), which also exhibited the strongest characteristic aroma compared to the other formulations.

**Table 2.** Organoleptic Characteristics of Ointment Containing a Combination of Mangkokan Leaf Extract (*Nothopanax scutellarium*) and Taro Leaf Stalk Extract (*Colocasia esculenta* L.)

| Ointment Formula | Form | Color | Aroma |
|---|---|---|---|
| **F0** | Semi-solid | White | Odorless |
| **F1** | Semi-solid | Light green | Characteristic aroma of mangkokan leaf extract |
| **F2** | Semi-solid | Light green | Characteristic aroma of mangkokan leaf extract |
| **F3** | Semi-solid | Light yellow | Characteristic aroma of mangkokan leaf extract |



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**Homogeneity Test of Ointment Preparations**

The homogeneity test was performed to determine whether the ointment preparations were uniformly mixed, indicating an even distribution of the active ingredients within the ointment base. This test also aimed to identify lumps or coarse particles that could potentially cause skin irritation. Based on the results of the homogeneity evaluation, it can be concluded that the blank preparation (F0), F1, F2, and F3 all met the requirements for homogeneity, indicating that the active ingredients and base were well blended in each formulation.

**Table 3.** Homogeneity Test Results of Ointment Formulations

| Ointment Formula | Cycle 1 (Day 1–3) | Cycle 2 (Day 4–6) | Cycle 3 (Day 7–9) |
|---|---|---|---|
| F0 | Homogeneous | Homogeneous | Homogeneous |
| F1 | Homogeneous | Homogeneous | Homogeneous |
| F2 | Homogeneous | Homogeneous | Homogeneous |
| F3 | Homogeneous | Homogeneous | Homogeneous |

**Spreadability Test of Ointment Preparations**

The spreadability test was carried out to assess the ability of the ointment containing the combined extracts of mangkokan leaves and taro leaf stalks to spread evenly on a flat glass surface. During the test, the ointment sample was placed between glass plates and subjected to additional loads of 50 g and 100 g. The results showed that the blank formulation had a spreadability diameter of 5.1 cm, Formula I 6.0 cm, Formula II 5.5 cm, and Formula III 5.2 cm. Among the tested formulations, Formula I demonstrated the best spreadability. Adequate spreadability is expected to influence the diffusion rate of the active compounds across biological membranes. A wider spreading area increases the diffusion coefficient, which facilitates greater drug diffusion. Therefore, a formulation with higher spreadability is generally considered more favourable.

**Table 4.** Spreadability Test Results of Ointment Preparations

| Ointment Formula | F0 | F1 | F2 | F3 | Standard Requirement |
|---|---|---|---|---|---|
| Cycle 1 (Day 1–3) | 4.5 cm | 6.0 cm | 5.5 cm | 5.2 cm | 5–7 cm |
| Cycle 2 (Day 4–6) | 5.5 cm | 5.2 cm | 5.3 cm | 5.0 cm | |
| Cycle 3 (Day 7–9) | 5.1 cm | 5.8 cm | 5.5 cm | 5.3 cm | |
| Average | 5.0 cm | 5.6 cm | 5.4 cm | 5.1 cm | |

**pH Test of Ointment Preparations**

The pH test was conducted to determine the acidity or alkalinity of the ointment preparations and to ensure that the formulations would not cause skin irritation or dryness when applied. The acceptable pH range for topical ointments is 4.5–6.5, which corresponds to the natural pH of human skin. The results indicated that the pH values of the tested ointment formulations fell within this acceptable range, suggesting that the preparations are safe and suitable for topical application.

**Table 5.** pH Test Results of Ointment Preparations

| Ointment Formula | F0 | F1 | F2 | F3 | Standard Requirement |
|---|---|---|---|---|---|
| **Cycle 1 (Day 1–3)** | 5.8 | 5.7 | 5.5 | 5.6 | 4.5–6.5 |
| **Cycle 2 (Day 4–6)** | 5.4 | 5.3 | 5.3 | 5.3 | |
| **Cycle 3 (Day 7–9)** | 5.2 | 5.5 | 5.7 | 5.8 | |
| **Average** | 5.4 | 5.5 | 5.5 | 5.3 | |

**Incision Wound Healing Activity**

The results indicate that all treatment groups experienced a gradual reduction in wound length over the observation period, demonstrating progressive healing. Among the tested formulations, Formula 1:2 showed the fastest wound closure, reaching complete healing earlier than the other treatment groups. The measurement of wound length presented in Table 5 indicates that all treatment groups showed progressive changes in wound size from day 1 to day 10. Both the table and graphical observations demonstrate a gradual reduction in incision length in each treatment group, indicating continuous healing during the



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observation period. Wound evaluation was performed daily until complete healing occurred. The criteria used to determine wound healing included the absence of redness and swelling and complete closure of the wound surface. When these indicators were fulfilled, the wound was considered healed [9].

**Table 6.** Average Measurement of Incision Wound Length in Male Rabbits from Day 0 to Day 10

| Treatment Group | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Negative Control | 1.2 | 1.0 | 0.9 | 0.8 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 |
| Positive Control (Betadine Ointment) | 1.2 | 1.0 | 0.9 | 0.8 | 0.7 | 0.5 | 0.4 | 0.2 | 0.1 | 0.0 | 0.0 |
| Formula 2:1 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 | 0.5 | 0.4 | 0.2 | 0.1 | 0.0 |
| Formula 1:1 | 1.2 | 1.0 | 0.9 | 0.8 | 0.6 | 0.5 | 0.4 | 0.2 | 0.0 | 0.0 | 0.0 |
| Formula 1:2 | 1.2 | 0.8 | 0.7 | 0.6 | 0.4 | 0.3 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |

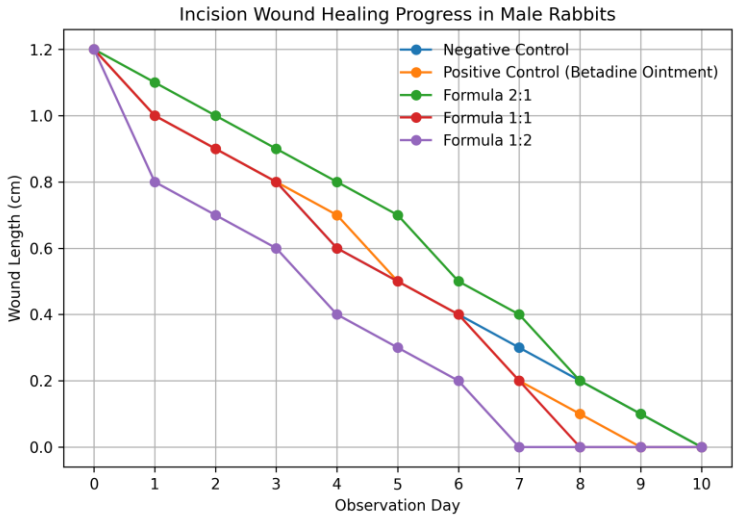


**Figure 1:** Development of Wound Healing Activity

**Table 7.** ANOVA Test Results

| Day | F Value | Sig. (p) | Interpretation |
|---|---|---|---|
| Day 1 | 3.600 | 0.046 | Significant |
| Day 2 | 3.600 | 0.046 | Significant |
| Day 3 | 3.600 | 0.046 | Significant |
| Day 4 | 6.600 | 0.007 | Significant |
| Day 5 | 6.000 | 0.010 | Significant |
| Day 6 | 3.600 | 0.046 | Significant |
| Day 7 | 8.250 | 0.003 | Significant |
| Day 8 | 5.000 | 0.018 | Significant |
| Day 9 | 2.250 | 0.136 | Not significant |
| Day 10 | — | — | Not analyzable (all wounds healed) |

The results of the one-way ANOVA test indicate that significant differences among treatment groups were observed from day 1 to day 8, as reflected by the significance values obtained from the ANOVA test ($p < 0.05$). No statistically significant difference was observed on day 9 ($p = 0.136$). This finding most likely reflects convergence of the healing process among treatment groups, as three groups had already achieved complete wound closure. In contrast, the remaining groups exhibited only minimal residual wounds. Consequently, the ability to detect treatment-related differences decreased substantially during the final stage of healing. This occurred because three treatment groups—positive control, F2, and F3—had already achieved complete wound healing, while the negative control and F1 groups were nearly healed. On day 10, the ANOVA analysis could not be performed because all treatment groups had reached the same wound length value (0 cm), indicating complete healing.

Formula F1 demonstrated the highest spreadability among the tested formulations. This finding may be attributed to its lower viscosity resulting from the balance between extract concentration and ointment


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base. Increasing the proportion of plant extracts may alter the internal structure of the semisolid system, thereby increasing viscosity and reducing spreadability. Although F1 exhibited the best spreading characteristics, spreadability alone does not necessarily determine therapeutic efficacy because wound healing also depends on the biological activity of the incorporated phytochemicals.

It should be noted that the present study did not perform accelerated stability testing according to ICH guidelines. The physical evaluations represented short-term observations under room-temperature storage conditions only. Therefore, long-term physicochemical stability of the ointment cannot yet be concluded. Further analysis using the Tukey HSD post-hoc test showed that significant differences mainly occurred between the negative control group and the combination extract formulations, as well as between the positive control group and the more effective formulations (F2 and F3).

Among the tested formulations, Formula F3 (5% mangkokan: 10% taro) demonstrated the fastest wound closure. One possible explanation is that the higher proportion of taro petiole extract provided greater concentrations of bioactive compounds, particularly flavonoids, saponins, and terpenoids, which have been reported to possess potent anti-inflammatory, antioxidant, antimicrobial, and collagen-stimulating activities. These phytochemicals may contribute to earlier resolution of inflammation and more rapid re-epithelialization. However, because the phytochemical constituents of each extract were not quantitatively standardised in the present study, this explanation remains hypothetical and requires further confirmation.

The ANOVA findings support the wound measurement results, which show that the combination ointment containing mangkokan leaf extract and taro leaf stalk extract accelerates incision wound healing. The Tukey post-hoc analysis further demonstrated that Formula F3 (5%: 10%) differed significantly from the negative control, positive control, and Formula F1, particularly between day 4 and day 8. In addition, Formula F2 (7.5%: 7.5%) also showed significant differences compared to the negative control on days 4, 5, and 8. These results indicate that the combination of mangkokan and taro extracts, especially in the F3 ratio, produced the strongest and fastest wound healing effect.

These findings are consistent with a previous study that evaluated the effectiveness of an ethanol extract cream formulation of taro leaf stalk in healing incision wounds in Wistar strain *Rattus norvegicus*. The study reported that the fastest wound healing occurred on day 14 at a concentration of 15% [10]. Similarly, a study by Revina et al. (2018) investigating the effectiveness of mangkokan leaf ointment in treating burn wounds in rats found that wound healing occurred on day 21 with concentrations of 50% and 75% [5].

The ointment combining extracts of mangkokan leaves and taro leaf stalks contains various secondary metabolites that contribute to wound healing activity. Taro leaf stalks contain several phytochemical compounds, including flavonoids, tannins, alkaloids, saponins, steroids, and terpenoids. Previous research by Erni Rustian et al. reported that taro leaf stalks contain 3.18 ± 0.0581% flavonoids and 7.10 ± 0.0676% terpenoids. These bioactive compounds play an important role in wound healing due to their antibacterial, anti-inflammatory, and antioxidant activities, while tannins function as astringent agents that help constrict skin pores, reduce exudate formation, and control minor bleeding. [1,6].

Flavonoids are polyphenolic compounds known for their anti-inflammatory and antibacterial properties. They exert antibacterial activity by forming complex compounds with extracellular proteins, which disrupt the integrity of bacterial cell membranes. In addition, flavonoids can stimulate wound repair processes such as angiogenesis, re-epithelialization, and collagen synthesis through the activation of signalling pathways including PI3K/Akt, TGF-β, Nrf2/ARE, and MAPK/ERK. [11].

Terpenoids exhibit antibacterial activity by interacting with porin proteins (transmembrane proteins) located in the outer membrane of bacterial cell walls. This interaction leads to the formation of strong polymeric bonds that damage the porin structure. Since porins function as channels for the transport of molecules across the bacterial membrane, their disruption reduces membrane permeability. It prevents nutrient uptake, ultimately inhibiting bacterial growth or causing bacterial cell death. [12]. Saponins also demonstrate strong antimicrobial activity by disrupting the stability of microbial cell membranes. Furthermore, saponins can enhance the expression of TGF-β receptors in fibroblasts, strengthening TGF-β binding and promoting fibroblast proliferation and collagen production, which are essential processes in wound healing. [13].

The combination of these two plant extracts may provide complementary biological activities that contribute to wound healing. However, because this study did not include groups treated with each extract individually, the presence of a true synergistic interaction cannot yet be confirmed. A study on native Australian medicinal plants reported that combining plant extracts synergistically increased antioxidant


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activity, activated the Nrf2 pathway, and improved fibroblast wound healing. These findings support the hypothesis that phytochemical combinations from different herbal sources may provide stronger biological effects than single-plant extracts [14]. From a practical perspective, these results highlight the potential development of synergistic herbal ointment formulations as alternatives to conventional antiseptics such as povidone-iodine. With its rapid wound healing activity and clearly supported biological mechanisms, the mangkokan–taro combination ointment may represent a promising natural therapy that is effective while remaining compatible with tissue healing processes.

However, several limitations should be considered. Future studies are recommended to include larger sample sizes (e.g., 5–6 animals per group), determined using established approaches such as the Federer formula ((t−1)(n−1) ≥ 15), to improve statistical robustness and external validity. The extraction yield of each plant material was not determined in the present study, and extract standardisation could not be evaluated quantitatively. Future studies should report extraction yield and phytochemical standardisation to improve reproducibility.

Although repeated daily observations provided longitudinal information regarding wound healing progression, the limited number of animals may have reduced statistical power and increased biological variability. Daily measurements were analysed independently using one-way ANOVA because the primary objective was to compare treatment groups at each observation day. However, this approach does not account for within-subject correlations arising from repeated measurements and may increase the probability of Type I error.

In addition, the evaluation relied primarily on macroscopic wound measurements without supporting histological analysis or molecular biomarker assessment. Although this method is commonly used in preclinical wound-healing studies, it cannot fully represent tissue generation occurring at the microscopic level. Future studies should integrate macroscopic assessment with histopathological parameters such as epithelial thickness and collagen deposition, and molecular biomarker analysis including VEGF and collagen type I expression to provide more comprehensive evidence regarding the mechanisms responsible for wound healing. The present findings should be interpreted as preliminary evidence rather than definitive confirmation of treatment efficacy.

## Conclusions

The combination of mangkokan leaf extract and taro leaf stalk extract was successfully formulated into an ointment preparation that fulfilled all physical quality parameters, including organoleptic characteristics, homogeneity, pH, and spreadability. All tested formulations demonstrated the ability to accelerate incision wound healing; however, the 5%: 10% formulation (F3) showed the highest effectiveness based on the results of the ANOVA and post-hoc analyses. Future studies are recommended to perform more comprehensive evaluations, such as measuring the depth of incision wounds, conducting histological analyses of tissue healing (including angiogenesis, collagen deposition, and epithelial thickness), and investigating the underlying biological mechanisms in greater detail to confirm the roles of flavonoids and saponins in the wound healing process.

## Conflict of Interest

There is no conflict of interest in this research.

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