ACTA VETERINARIA INDONESIANA

Vol. 14, No. 1: 96-102, March 2026
DOI : 10.29244/avi.14.1.96-102
P-ISSN 2337-3202, E-ISSN 2337-4373

Research Article

OPEN ACCESS

Evaluation of the Effectiveness of Agathis Ointment on Wound Healing Incisions in Rats (*Rattus Norvegicus*)

Andi Muhammad Ridha Hafizh[1], Gunanti[2*], Rini Madyastuti Purwono[3], Arni Diana Fitri[4], Andy[1], Akbar Muzaky[1]

[1]Graduate Program, School of Veterinary Medicine and Biomedical Sciences, IPB University, Jln. Agathis Kampus IPB Darmaga, Bogor 16680, Indonesia
[2]Division of Surgery, Animal Biomedical Sciences, IPB University, Jln. Agathis Kampus IPB Darmaga, Bogor 16680, Indonesia
[3]Division of Veterinary Pharmacy, Animal Biomedical Sciences, IPB University, Jln. Agathis Kampus IPB Darmaga, Bogor 16680, Indonesia
[4]Educational Animal Hospital Staff, IPB University, Jln. Agathis Kampus IPB Darmaga, Bogor 16680, Indonesia

*Corresponding author: gunantisoe@apps.ipb.ac.id

Article history:
Received: 9 July 2025
Revised : 25 November 2025
Accepted: 23 February 2026

Keywords:
agathis ointment
wound healing
rats
inflammatory cells
topical treatment

ABSTRACT

Background: Wound healing is a complex biological process involving the phases of inflammation, proliferation, and tissue remodeling. Various topical agents have been developed to accelerate this process by reducing inflammation, preventing infection, and promoting tissue regeneration.

Aims: This study aimed to evaluate the effectiveness of Agathis ointment in accelerating excisional wound healing in rats (*Rattus norvegicus*).

Methods: A total of 18 male Sprague Dawley rats were divided into three treatment groups: a negative control group (no treatment), a positive control group (treated with commercial gentamicin ointment), and a treatment group (treated with Agathis ointment). Excisional wounds were created using a 5 mm punch biopsy tool, and treatments were applied twice daily for 10 days. Macroscopic observations were conducted by measuring wound area on days 3, 7, and 10, while microscopic observations included histological evaluation of inflammatory cell counts.

Results: TThe results showed that Agathis ointment significantly ($p>0.05$) reduced wound area and inflammatory cell numbers, particularly on day 10, with outcomes comparable to or better than the commercial ointment group. This effectiveness is presumed to result from the synergistic effects of active compounds in Agathis ointment, such as acetylsalicylic acid, gentamicin, bioplacenton, lever-tran, and Peru balsam, which possess anti-inflammatory, antimicrobial, and tissue regeneration-promoting properties.

Conclusion: In conclusion, Agathis ointment shows promising potential as an effective topical agent for accelerating wound healing in experimental animal models.

INTRODUCTION

The skin is the outermost organ that protects the body and plays an important role in maintaining internal balance (homeostasis) by regulating body temperature, protecting against external damage, and responding to various stimuli (Endorgan 2021).

The wound healing process involves four main phases, namely homeostasis, inflammation, proliferation, and restructuring. This process involves cooperation between skin cells and the matrix, and is supported by various components such as platelets, keratinocytes, immune cells, microvascular cells, and fibroblasts. Wound care includes a series of actions such as dressing, the use of painkillers, anti-inflammatory agents, antimicrobials, and medications to support healing. Recent research continues to develop the use of synthetic and biological materials for more effective wound management (Primadina 2019).

Agathis ointment is a compound ointment used in the healing process of wounds at RSHP. It is commonly used for wound healing. Agathis ointment is a compound preparation containing acetylsalicylic powder, gentamicin, levertranz, peru balsam, and Biopasenton®. Wound healing is a complex biological process involving various phases, including hemostasis, inflammation, proliferation, and maturation. In veterinary medicine, wound management in animals is crucial to prevent infection, accelerate recovery, and reduce the risk of further complications.

MATERIALS AND METHODS

Research Materials

Test animals

The test animals used were adult male Sprague Dawley rats weighing 200-250 g in good health. The rats were fed a standard diet. Water was provided ad libitum, and the rats were kept in normal environmental conditions at a temperature of 21 °C and a humidity of 50-60%.

Agathis Ointment

Agathis Ointment was obtained from the Educational Animal Hospital (RSHP) SKHB IPB University with the contents of acetylsalicylic powder, gentamicin, levertranz, peru balsam, and Biopasenton ®.

Research Method

This research has been approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences (SKHB), IPB University with number: 327/KEH/SKE/IV/2025. Furthermore, the research stages are described as follows.

Division of Rat GROUPS

A total of 18 test animals were divided into 3 treatment groups, with 6 rats in each group. The Negative group consisted of injured rats that were not treated, serving as the negative control. The Positive group consisted of injured rats that were treated with commercial ointment, serving as the positive control. The Agathis group consisted of injured rats that were treated with Agathis ointment.

Wound Treatment

The rats were anesthetized with 10% Ketamine and 2% Xylazine, then the hair was shaved on the back area to be excised. The excision wound was made on the back of the rat using a sterile technique, namely a 5 mm diameter biopsy punch with a depth up to the subcutis. The wound treatment groups were first cleaned with normal saline solution, then dried with sterile gauze. The negative control group was not treated with ointment, while the positive control group was treated with commercial ointment (Genoint ®) and the treatment control group was treated with Agathis ointment. Each group was treated twice a day for 10 days.

Macroscopic and microscopic observations

Macroscopic observations to evaluate wound healing were performed by measuring the wound area and taking photographs of the wound condition. Measurements and observations of the wound were performed for each animal on days 3, 7, and 10 and after skin incision. Microscopic observation with punch biopsy of the experimental rat skin was performed on days 3, 7, and 10 after treatment.

Microscopic observation of anesthetized mice was performed using a combination of ketamine at a dose of 10 mg/kgBW and xylazine at a dose of 2 mg/kgBW intraperitoneally. The mice were then necropsied, and skin samples were taken from the 5 mm punch biopsy area. The skin on the back was first cleaned of hair that had begun to grow and trimmed to a thickness of ± 3 mm. The skin obtained was placed in 10% Neutral Formalin Buffer (BNF), then the samples underwent dehydration by soaking the specimens in 70%, 80%, 90% alcohol, absolute alcohol, xylene, and paraffin. Each preparation was soaked for 2 minutes, followed by printing and tissue cutting using a microtome with a thickness of 4-5 µm. The cutting results were placed on a glass slide, which was then dried in a 60°C incubator. The preparations were then stained with Hematoxylin-Eosin (HE) and observed under a light microscope. The parameters observed included the calculation of the number of fibroblasts, neovascularization, inflammatory cells, and re-epithelialization with 10 fields of view.

Data Analysis

The results of anatomical pathology observations were analyzed using ImageJ and Microsoft Excel applications. All quantitative data from this study were statistically analyzed using R software ver.3.5.3 with the one-way ANOVA analysis method. Before conducting the One Way ANOVA test, normality and homogeneity tests were performed. Then, the Tukey test was performed if there were significant differences in the results. Data were presented in the form of means and standard deviations and described descriptively.

RESULTS AND DISCUSSION

Measurement of Wound Area

The data from the wound healing process observations are presented in Tables 1 and Figure 1 below.

Table 1. Avarage total wound area (cm)

Group	Day		
	3	7	10
KN	0.54 ±0.08[a]	0.41 ± 0.12[a]	0.22 ± 0.06[a]
KP	0.49 ± 0.08[a]	0.18 ±0.09[b]	0.11 ± 0.02[b]
AG	0.52 ± 0.08[a]	0.23 ± 0.13[ab]	0.07 ± 0.02[b]

Note: KN = negative control; KP = positive control; AG = Agathis control; Different superscript letters in the same column indicate significant differences at the 5% test level.

Macroscopic area measurements (Table 1) based on macroscopic wound area measurements showed that in the KN group, wound size decreased gradually from day 3 (0.54 ± 0.08[a]) to day 7 (0.41 ± 0.12[a]) to day 10 (0.22 ± 0.06[a]), but there was no significant difference between days, indicating a slow healing process. The KP group showed a faster decrease in wound size from 0.49 ± 0.08[a] (day 3) to 0.18 ± 0.09[b] (day 7) and 0.11 ± 0.02[b] (day 10), indicating the effectiveness of the ointment in significantly accelerating wound healing compared to KN. The AG group showed a decrease in wound size from 0.52 ± 0.08[a] (day 3) to 0.23 ± 0.13[ab] (day 7) and 0.07 ± 0.02[b] (day 10). These results indicate that Agathis ointment is also effective in accelerating wound healing, which is particularly significant on day 10, with results comparable to KP and better than KN.

The results of the wound area observations presented (Figure 1) show that on day 3, all groups had wounds that were still wide open, round in shape, with scabs, very clear wound edges, and no signs of wound closure. On day 7, the KN group had wounds with edges that were still wide but with slow scab formation. The KP group showed that the wound had started to shrink compared to day 3, with a little scab and dryness, but the AG group showed a reduction in the size of the wound area compared to days 3 and 7, with the wound starting to dry and new tissue starting to form around it.

On day 10, the wounds in the KN group appeared to have shrunk compared to day 7, but were still open and not yet completely closed. The wounds in the KP group were almost completely closed, with clear signs of tissue regeneration indicating the effectiveness of KP administration, while the wounds in the AG group were almost completely closed and healed, with the wound surface shrinking to a small dot.

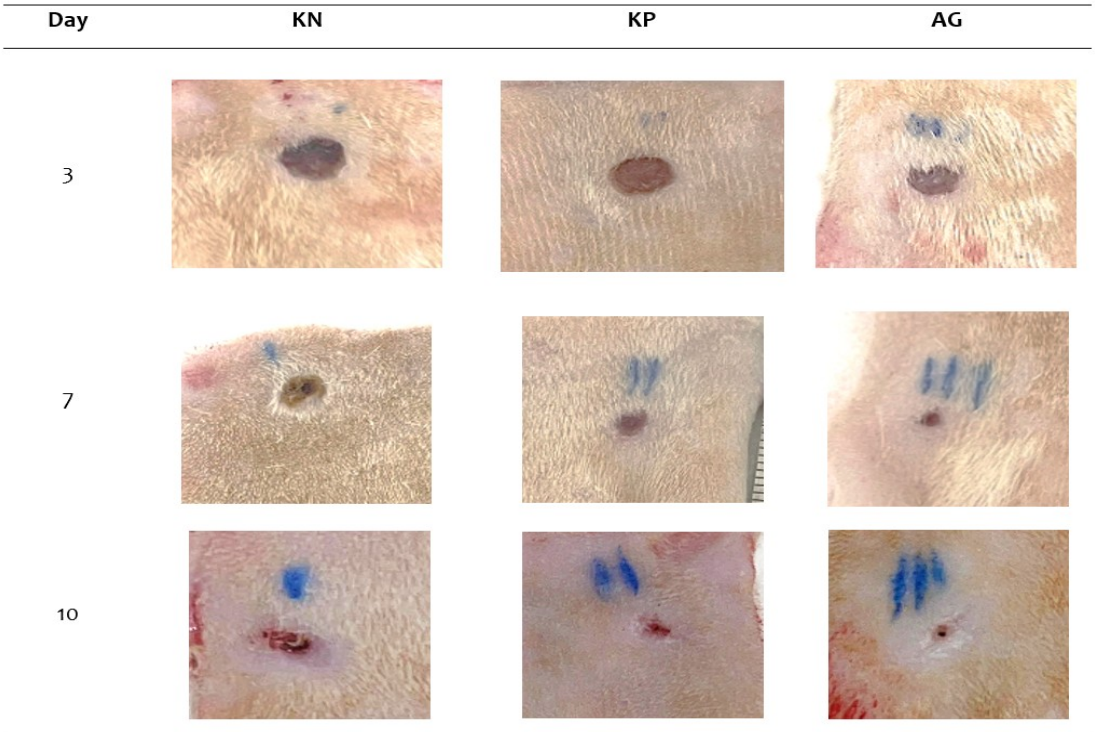

Figure 1. Observation of Wound Area.

Microscopic Observation

Inflammatory cells are part of the immune system that plays an important role in the inflammatory response as the body's defense mechanism against traumatic injuries, irritation, or infection. The average number of inflammatory cells observed in the tissue can be seen in Table 2.

Table 2. Average inflammatory cells on days 3, 7, and 10

Group	Day		
	3	7	10
KN	92.6 ± 17.90^{a}	133.9 ± 77.29^{a}	58.5 ± 8.66^{a}
KP	84.3 ± 34.77^{a}	82.9 ± 51.84^{ab}	38.5± 11.76^{b}
AG	61.6 ± 31.34^{a}	35.7 ± 6.36^{b}	24.1 ± 2.74^{b}

Note: KN = negative control; KP = positive control; AG = Agathis control; Different superscript letters in the same column indicate significant differences at the 5% test level.

The number of inflammatory cells observed in the wound tissue (Table 2) showed a downward trend from day 3 to day 10 in all treatment groups. On day 3, all groups showed high numbers of inflammatory cells, namely 92.6 ± 17.90^{a} (KN), 84.3 ± 34.77^{a} (KP), and 61.6 ± 31.34^{a} (AG). On day 7, differences began to appear in the AG group, showing the largest decrease in the number of inflammatory cells (35.7 ± 6.36^{b}), which was significantly different from KN (133.9 ± 77.29^{a}) and KP (82.9 ± 51.84^{ab}). This indicates that Agathis ointment is able to reduce the inflammatory process more quickly than the other groups. On day 10, the AG group still showed the lowest number of inflammatory cells (24.1 ± 2.74^{b}), significantly lower than KN (58.5 ± 8.66^{a}) and KP (38.5 ± 11.76^{b}). These results indicate that Agathis ointment has a strong anti-inflammatory effect and accelerates the resolution of inflammation during the wound healing process.

The graph (Figure 2) shows a downward trend in the number of inflammatory cells from day 3 to day 10 in all treatment groups. On day 3, all groups (KN, KP, and AG) had high numbers of inflammatory cells that were relatively insignificant. On day 7, there was a noticeable difference, with the AG group showing the most drastic decrease in the number of inflammatory cells, while KN remained high and KP experienced a slower decrease. On day 10, the AG group recorded the lowest number of inflammatory cells, indicating the anti-inflammatory effectiveness of Agathis ointment was better than KN and KP. Overall, this graph supports that Agathis ointment accelerates inflammatory resolution during wound healing, with a significant and consistent decrease in inflammatory cells throughout the observation period.

Wound healing is a complex biological process involving various types of cells, including fibroblasts, inflammatory cells, new blood vessel formation, and re-epithelialization in each phase of wound healing. In the early phase of healing, there is an important interaction between fibroblasts and immune cells (Cial-dai et al., 2022). The effectiveness of a topical agent in wound healing can be observed through a macroscopic reduction in wound area during the

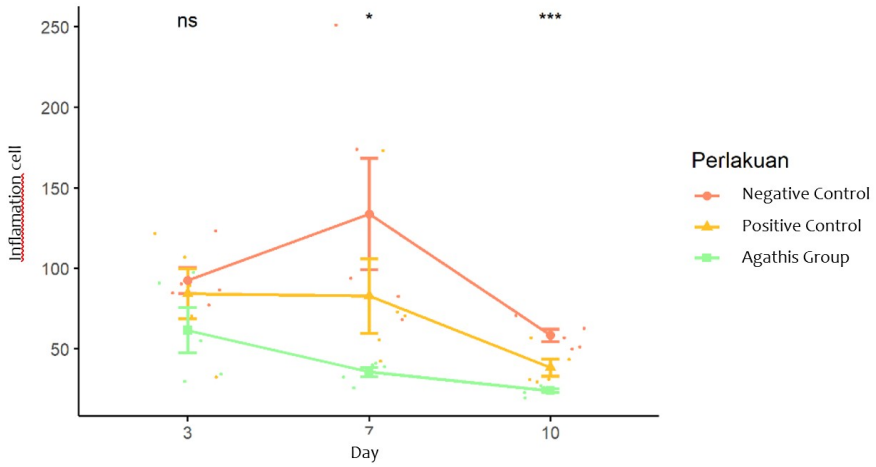

Figure 2. Total inflammatory cell count on days 3, 7, and 10; ns = not significant; one star = significant (p<0.05); three stars = significant (p<0.001).

observation period. Macroscopic evaluation of the experimental rat skin was observed by calculating the wound area (cm). Macroscopic measurement of wound area is a key indicator in assessing the speed of wound healing. The decrease in wound size over time visually reflects the success of the healing process, starting from the inflammatory phase, proliferation, and remodeling. If the ointment administered is effective, the wound area will decrease significantly in a shorter time (Li et al., 2022).

The evaluation in this study was conducted on days 3, 7, and 10 after treatment, with results that provided a progressive picture of the ability of each treatment to accelerate the wound healing process. Observations in the KN group showed a decrease in wound area on days 3, 7, and 10. The insignificant decrease on days 3, 7, and 10 indicates that natural wound healing without topical intervention is slow (Yen et al., 2018). This is most likely due to limited stimulation of the proliferative process and the slow tissue remodeling phase without the help of active substances (Nour et al., 2019). The KP group administered with commercial ointment (Gentamicin) showed a faster and significant reduction in wound area on days 3, 7, and 10. These results indicate that the topical preparation used in the KP group is effective in accelerating the wound healing process, primarily through accelerated reepithelialization and reduced inflammation (Nikolic et al., 2025). The AG group showed a similar, even better response on day 10.

The reduction in wound area from day 3 to day 10 shows that Agathis ointment is able to significantly accelerate wound healing, especially in the final phase of observation. These results are even better than KN and comparable to KP, indicating that Agathis ointment has promising therapeutic potential. The effectiveness of Agathis ointment in accelerating wound healing is supported by its active ingredient composition, namely Acetyl Salicylic Powder, Gentamicin, Bioplacenton, Levertran, and Peru balsam. Acetyl Salicylic Powder has anti-inflammatory effects that suppress pro-inflammatory mediators such as prostaglandins (Le et al., 2021). Gentamicin provides protection against bacterial infections that can slow down tissue regeneration (Wang et al., 2019). Bioplacenton, which contains placenta extract, is rich in growth factors that stimulate the migration and proliferation of fibroblasts and keratinocytes (Almas et al., 2020). Levertran (fish oil) contains vitamins A and D that support epithelialization, while Peru balsam increases local blood perfusion and has antiseptic and promotive effects on new tissue formation (Chan 2018). With the synergy of these ingredients, Agathis

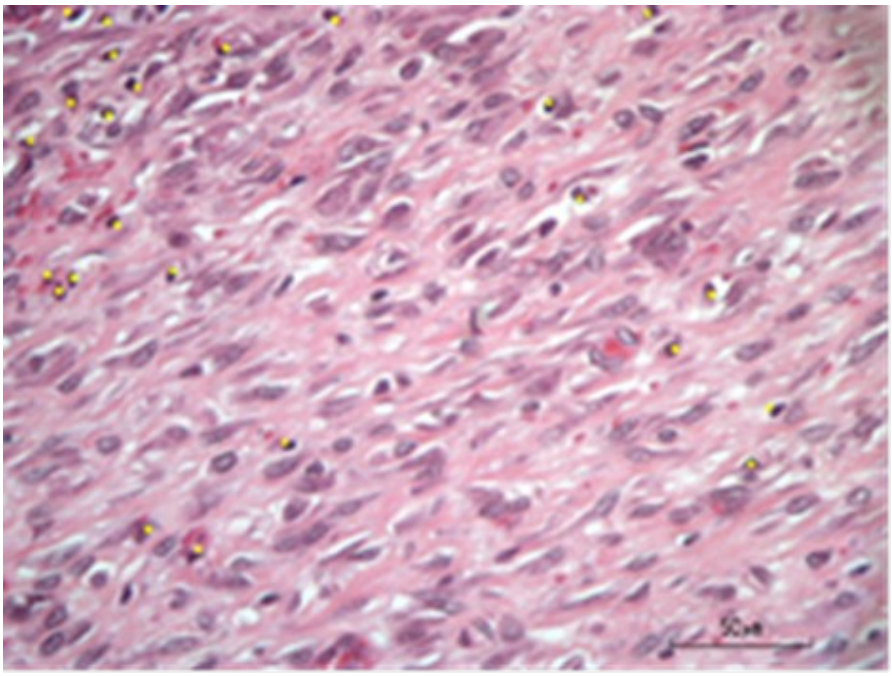

Figure 3. Histopathology of rat skin in the Agathis treatment group on day 10, HE staining; yellow dots = inflammatory cells.

ointment creates a microenvironment in the wound that supports optimal healing, both through reducing inflammation, preventing infection, and directly stimulating the regenerative process.

The wound healing process begins with an inflammatory phase characterized by the infiltration of inflammatory cells, such as neutrophils and macrophages, into the wound area (Ellis and Tartar 2018). This phase aims to cleanse the tissue of pathogens and debris, but prolonged inflammation can hinder the healing process. Rapid inflammation is an important indicator of efficient wound healing (Ridiandries et al 2018). Based on the results of the study, the number of inflammatory cells showed a decrease from day 3 to day 10 in all treatment groups.

On day 3, the number of inflammatory cells remained high in all three groups without any statistically significant differences. This reflects a normal acute inflammatory response following tissue injury caused by biopsy (Govindaraju et al., 2019). On day 7, differences began to appear in the AG group, showing the most significant decrease in the number of inflammatory cells compared to the KN and KP groups. This significant decrease indicates that Agathis ointment has a faster anti-inflammatory effect than the control groups, both negative and positive. This effect is influenced by the active ingredients in Agathis ointment, such as acetylsalicylic acid, which has anti-inflammatory properties (Kuli-kova et al., 2020), and peru balsam, which is known to have immunomodulatory and antimicrobial effects (Aguiar et al., 2022).

On day 10, the AG group continued to show the lowest number of inflammatory cells, which was lower than the other groups. The consistency of these results strengthens the evidence that Agathis ointment plays a role in accelerating the resolution of inflammation, which is an important prerequisite for transition to the proliferative phase. This process is important because prolonged inflammation can inhibit fibroblast activity and interfere with granulation tissue formation (Alhajj and Goyal 2022).

Agathis ointment has anti-inflammatory effects, as demonstrated by a faster and more consistent reduction in the number of inflammatory cells compared to the control. This supports the therapeutic role of Agathis ointment in accelerating wound healing through the control of the inflammatory response from an early stage.

CONCLUSION

From the results of this study, it can be concluded that Agathis ointment is highly effective in accelerating the wound healing process in a rat biopsy wound model. This effectiveness is demonstrated by a faster decrease in the number of inflammatory cells in the Agathis treatment group compared to the control group, indicating that the inflammatory phase is shorter.

AUTHORS CONTRIBUTION

A.M.R.H. carried out the experimental work, collected and analyzed the data, and prepared the initial draft of the manuscript. A.D.F., A., and A.M. assisted in laboratory procedures, animal handling, and data collection during the study. R.M.P. contributed to the study design, interpretation of the findings, and provided critical input in manuscript revision. G. served as the principal investigator and corresponding author, overseeing the research process, guiding the experimental design, and reviewing the manuscript. All authors read and approved the final manuscript.

"The author declares that there is no conflict of interest with the parties involved in this research".

REFERENCES

Aguiar, A. T. C., Barcellos-Silva, I. G. C., de Oliveira Habib-Pereira, N. R., Antonio, A. S., da Veiga-Junior, V. F. (2022). Chemistry, biological activities, and uses of balsams. In: *Gums, Resins and Latexes of Plant Origin: Chemistry, Biological Activities and Uses*. Springer International Publishing. Cham. p399-432.

Alhajj, M., Goyal, A. (2022). Physiology, granulation tissue. In: *StatPearls [Internet]*. StatPearls Publishing.

Almas, A. I., Purnawati, R. D., Istiadi, H., Susilaningsih, N. (2020). The effect of honey in second degree burn healing on Wistar rats (overview of angiogenesis and the number of fibroblasts). *J Med Heal*, 11, 27-32.

Chan, O. W. K. (2018). Effect of fish oil application and its combined effect with therapeutic ultrasound on tendon healing: a rat model. Tesis S2.

Cialdai, F., Risaliti, C., Monici, M. (2022). Role of fibroblasts in wound healing and tissue remodelling on earth and in space. *Bioengineering and Biotechnology*, 1-18.

Ellis, S., Lin, E. J., Tartar, D. (2018). Immunology of wound healing. *Current Dermatology Reports*, 7, 350-358.

Erdogan, S. S., Gur, T. F., Terzi, N. K., Dogan, B. (2021). Evaluation of the cutaneous wound healing potential of tamanu oil in wounds induced in rats. *Journal of Wound Care*, 30(9), vi-vx.

Govindaraju, P., Todd, L., Shetye, S., Monslow, J., Puré, E. (2019). CD44-dependent inflammation, fibrogenesis, and collagenolysis regulates extracellular matrix remodeling and tensile strength during cutaneous wound healing. *Matrix Biology*, 75, 314-330.

Kulikova, O. I., Stvolinsky, S. L., Migulin, V. A., Andreeva, L. A., Nagaev, I. Y., Lopacheva, O. M., Fedorova, T. N. (2020). A new derivative of acetylsalicylic acid and carnosine: synthesis, physical and chemical properties, biological activity. *DARU Journal of Pharmaceutical Sciences*, 28, 119-130.

Le, N. P. K., Herz, C., Gomes, J. V. D., Forster, N., Antoniadou, K., Mittermeier-Kleßinger, V. K., Lamy, E. (2021). Comparative anti-inflammatory effects of Salix cortex extracts and acetylsalicylic acid in SARS-CoV-2 peptide and LPS-activated human in vitro systems. *International Journal of Molecular Sciences*, 22(13), 6766.

Li, L., Sun, Y., He, H., He, G., Ma, S., Yang, W., Wang, Y. (2022). Quantitative assessment of angiogenesis in skin wound healing by multi-optical imaging techniques. *Frontiers in Physics*, 10, 894901.

Nikolić, A., Milošević, I., Janković, A., Prokić, B. B., Nićković, E., Marković, D., Božinovski, T. L. (2025). Hydrogels poly (vinyl alcohol)/gentamicin and poly (vinyl alcohol)/chitosan/gentamicin: a promising approach to accelerate burn wound healing.

Nour, S., Baheiraei, N., Imani, R., Khodaei, M., Alizadeh, A., Rabiee, N., Moazzeni, S. M. (2019). A review of accelerated wound healing approaches: biomaterial-assisted tissue remodeling. *Journal of Materials Science: Materials in Medicine*, 30, 1-15.

Primadina, N., Basori, A., Perdanakusuma, D. S. (2019). Proses penyembuhan luka ditinjau dari aspek mekanisme seluler dan molekuler. *Qanun Medika*, 3(1), 31-43.

Ridiandries, A., Tan, J. T., Bursill, C. A. (2018). The role of chemokines in wound healing. *International Journal of Molecular Sciences*, 19(10), 3217.

Wang, P., Long, Z., Yu, Z., Liu, P., Wei, D., Fang, Q., Wang, J. (2019). The efficacy of topical gentamycin application on prophylaxis and treatment of wound infection: a systematic review and meta-analysis. *International Journal of Clinical Practice*, 73(5).

Yen, Y. H., Pu, C. M., Liu, C. W., Chen, Y. C., Chen, Y. C., Liang, C. J., Chen, Y. L. (2018). Curcumin accelerates cutaneous wound healing via multiple biological actions: the involvement of TNF-α, MMP-9, α-SMA, and collagen. *International Wound Journal*, 15(4), 605-617.