JPPIPA 10(12) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Healing Potential of *Chromolaena odorata* Extract: Modulation of Neutrophil, Macrophage, and Lymphocyte Response in Infected Wounds of *Mus musculus*

Melissa Abigail Yanis[1,2*], Mirnasari Amirsyah[1,2], Anastasia Dessy Harsono[3]

[1] Plastic Recontructive and Aesthetic Surgery Subdivision, Department Surgery, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[2] Zainoel Abidin General Hospital, Banda Aceh, Indonesia.
[3] Plastic Recontructive and Aesthetic Surgery Subdivision, Department Surgery, Gatot Suebroto Army Central Hospital, Jakarta, Indonesia.

Received: August 11, 2024
Revised: October 13, 2024
Accepted: December 25, 2024
Published: December 31, 2024

Corresponding Author:
Melissa Abigail Yanis
melissaabigaily@gmail.com

DOI: 10.29303/jppipa.v10i12.9423

Abstract: *Chromolaena odorata* has been reported to possess antimicrobial, antibacterial, and anti-inflammatory properties that aid in wound healing. Previous studies demonstrated the significant effects of a 7.5% concentration of *C. odorata* extract on non-infected wounds. This study aims to investigate its impact on infected wound healing. An experimental study was conducted on 36 mice, divided into two groups. Partial-thickness wounds were created on the dorsal side of each mouse and contaminated with *S. aureus* (106 CFU/ml). Group 1 was treated with normal saline, while Group 2 was treated with *C. odorata* aqueous extract at 7.5%. Histopathological analysis was performed on days 3 and 5 to count neutrophils, macrophages, and lymphocytes. Results showed a significantly lower neutrophil, macrophage, and lymphocyte count in Group 2 compared to Group 1 ($p < 0.05$). On day 3 and 5, Group 2 had lower neutrophil counts (5.6 ± 1.8, 5.8 ± 0.6) compared to Group 1 (9.2 ± 5.2, 15.1 ± 3.9). Macrophage counts were also lower in Group 2 (6.9 ± 1.7, 1.9 ± 0.7) compared to Group 1 (3.7 ± 1.4, 4.3 ± 0.9). Lymphocyte counts followed a similar trend, with Group 2 having fewer lymphocytes on both days ($p < 0.05$). In conclusion, *C. odorata* aqueous extract at a 7.5% concentration demonstrates potential as a wound healing agent by reducing inflammatory cells in infected wounds.

Keywords: *Chromolaena odorata*; Lymphocyte; Macrophage; Neutrophil; Wound healing

Introduction

Wounds are defined as an anatomical and functional disruption of the skin or deeper into the subcutis with damage to tendons, muscles, blood vessels, nerves, and even bones. Wounds can be caused by the discontinuity of tissue with a scalpel (incision) to extensive tissue damage (e.g. extensive trauma or burns). Wounds can also be the result of contusions, hematomas, lacerations, or abrasions (Enoch & Leaper, 2008).

Based on the healing time process, wounds are divided into acute and chronic wounds (Dagher et al., 2020; Raziyeva et al., 2021). Acute wounds such as trauma wounds, are wounds that progress according to the normal wound healing time. While chronic wounds failed to progress normally (Velnar et al., 2009; Falanga et al., 2022). Several factors that contribute to this failure are infection, tissue hypoxia, necrosis, and exudates.

Trauma is one of most common cause of wounds. According to the WHO, trauma cases result in at least six million deaths and tens of millions of admissions a year (Dai et al., 2011). Complications resulting from trauma

How to Cite:
Yanis, M. A., Amirsyah, M., & Harsono, A. D. (2024). Healing Potential of Chromolaena odorata Extract: Modulation of Neutrophil, Macrophage, and Lymphocyte Response in Infected Wounds of Mus musculus. *Jurnal Penelitian Pendidikan IPA*, *10*(12), 10670–10676. https://doi.org/10.29303/jppipa.v10i12.9423

wounds increase morbidity, mortality, and contribute to increased costs, care and length of stay (Cañedo-Dorantes & Cañedo-Ayala, 2019; Geraerds et al., 2019; Stokes et al., 2022).

Infection is the most common complication of trauma wounds. Trauma-related infections occur due to contamination of toxins, debris, and foreign bodies from the surrounding environment (Akiki & Mehrzad, 2020). Inadequate wound care and cleansing is also one of the causes of infection in trauma wounds (Tottoli et al., 2020). Trauma-induced infection is one of the factors that interfere with the wound healing process (Hamphanphoom et al., 2016; Becker et al., 2023).

Wound healing is a dynamic and complex biological process with the aim of restoring tissue integrity. The wound healing process generally occurs in four phases; hemostasis, inflammation, proliferation, and remodeling phases, which involve coordinated interactions of various immunological systems and these phases overlap with each other (Yang et al., 2021; Rodrigues et al., 2019). The wound healing process begins at the onset of the wound and continues through (Aziz, 2020; Tottoli et al., 2020).

Figure 1. *Chromolaena odorata*

Chromolaena odorata (*C. odorata*) is a plant that can thrive in most types of soil, commonly found in open fields and along roadsides (Figure 1). *C. odorata* is a plant that was originally discovered in South and Central America, which then spread to tropical and subtropical areas. Several chemical analyses of *C. odorata* have identified its contents, such as: monoterpenes, sesquiterpenes hydrocarbons, triterpenes/steroids, alkaloids, and flavonoids. The leaves of this plant are rich in flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempherol, and salvagenin. Extracts of the leaves and other parts of *C. odorata* have been reported to be beneficial in the wound healing process by modulating one or more phases of wound healing. *C. odorata* stimulates keratinocyte migration, enhances the formation of extracellular matrix proteins and basal membranes, and inhibits collagen contraction by fibroblasts. Therefore, this research was conducted to determine the activity of *C. odorata* extract in the healing of infected wounds (Yuliani & Viktor, 2015; Aziz et al., 2020; Olawale et al., 2022).

Method

Preparation of Chromolaena odorata Water Extract

C. odorata was obtained from agricultural land in Calang, Aceh Jaya. Fresh leaves are washed thoroughly with running water and dried in a clean environment. The dried leaves are ground and mixed with distilled water in a 1:2 ratio. After 24 hours, the macerate is separated and the residue is soaked again with aquadest for 24 hours. The entire process is repeated to ensure its completeness. Macerate 1, 2, and 3 were collected, filtered, and centrifuged at 5000 g and 4 °C for 30 minutes. After centrifugation, the obtained supernatant was frozen at -20 °C and then lyophilized. The extract is then filtered using Whatmann filter paper, and the filtrate is evaporated using a rotary evaporator to achieve the desired concentration of the extract (Hanh et al., 2011).

Animal Treatment

Thirty-six test animals have been divided into 2 treatment groups. After being adapted for one week, the test animals were subjected to wounds on their backs. Starting with shaving the dorsal part of the mouse with an area of 1.5 x 1.5 cm, followed by disinfection of the shaved back area using 70% alcohol. Next, the test animal was anesthetized using 75 mg/kg Ketamine and 15 mg/kg xylazine intramuscularly in the thigh and breathed spontaneously during the procedure. 24 partial thickness wounds or abrasions were created by scraping with a 24 scalpel ten times in one direction and ten times in the opposite direction, until redness or blood spots appeared. The wound was then contaminated with 0.02 mL of a suspension containing *S. Aureus* 10^6 colony forming units (CFU)/ml, 30 minutes after the injury. Each treatment group then received the following treatments: Group I (positive control), the test animals were subjected to a wound on the dorsal side, contaminated with *S. aureus*, and then cleaned with normal saline solution. Group II (experimental), the test animals were subjected to a wound on the dorsal side, contaminated with *S. aureus*, and then cleaned with a 7.5% concentration of *C. odorata* water extract solution.

Techniques for Examining Neutrophils, Macrophages, and Lymphocytes

Examination of Neutrophils, Macrophages, and Lymphocytes was conducted on the third and fifth days after the injury by taking skin samples for

histopathological examination. The technique for preparing histopathological specimens under a microscope involves taking skin tissue from the dorsal side of a rat, sized 3x3 cm. After that, the skin tissue is fixed using 10% Neutral Buffered Formalin to preserve the tissue structure. Next, the tissue undergoes a dehydration process with ethanol and is clarified using xylene. The tissue is then soaked in xylene embedding solution for 12 hours to prepare it before being placed into paraffin blocks. After that, the tissue is cut using a microtome with a thickness of 5 µm and assigned a protocol number for further identification. The staining process is carried out using Hematoxylin-Eosin to visualize the tissue structure under the microscope. Finally, the stained tissue was observed and counted under a microscope at 400x magnification (Braimah et al., 2017).

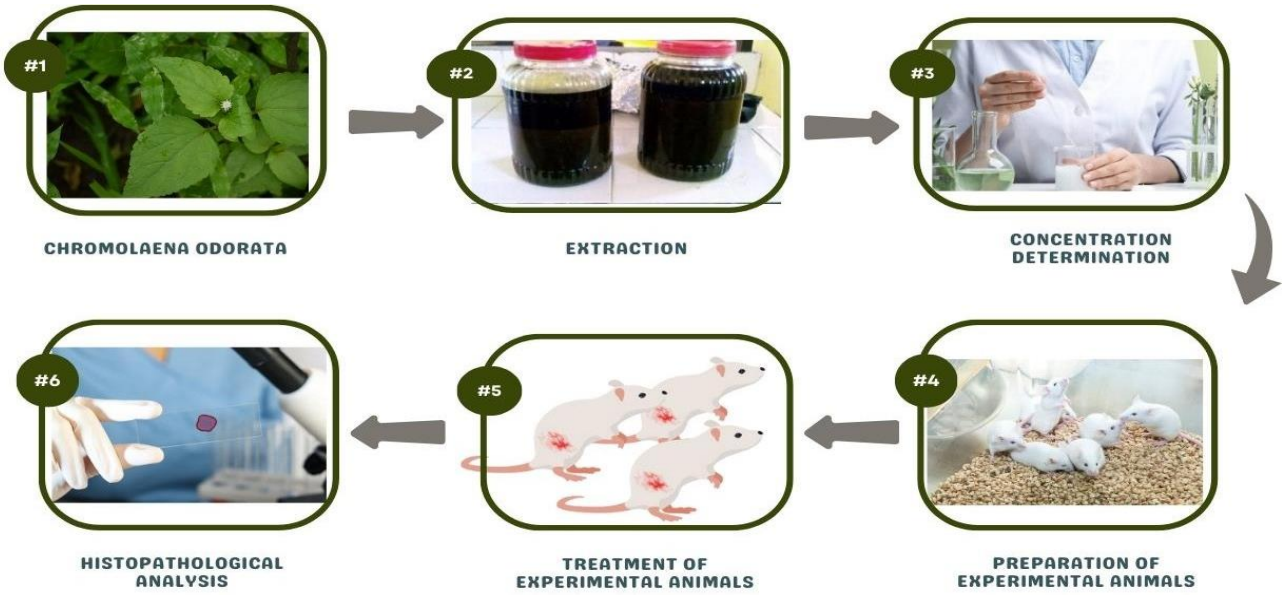

Figure 2. The schematic research procedure

Result and Discussion

Histopathological staining with Hematoxylin Eosin revealed that neutrophils are characterized as amphophilic cells with multilobed nuclei and purplish cytoplasmic granulation. Macrophages are larger than neutrophils, with irregularly shaped single nuclei and granule-free cytoplasm (Li et al., 2022; Shukla et al., 2019; Schreml et al., 2010). Meanwhile, lymphocytes are described as amphophilic cells with single round nuclei and clear cytoplasm as shown in Figure 2.

The result in Table 2, the mean neutrophil levels in the group given *C. odorata* aqueous extract concentration of 7.5% were significantly lower compared with the group given normal saline on the third and fifth day. Besides, the mean macrophage and lymphocytes levels in the group given *C. odorata* a water extract concentration 7.5% are higher compared to the group given normal saline on the third day. However, in the fifth day, the mean macrophage levels in the group given *C. odorata* water extract concentration 7.5% decreased, while the mean macrophage levels in the group given normal saline increased (Almadani et al., 2021; Gushiken et al., 2021; Guo & DiPietro, 2010).

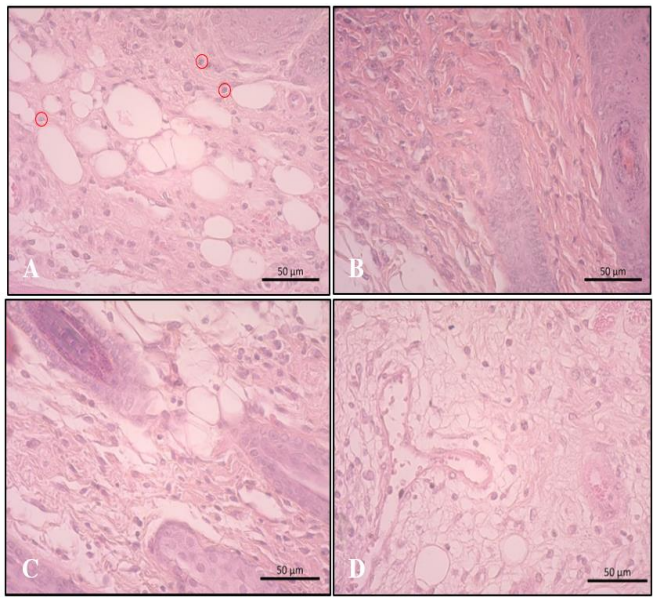

Figure 3. Histological tissue network of wound tissue staining with Hematoxylin Eosin. A) Experimental group with 7.5% concentration of *C. odorata* extract on the third day of observation, B) Experimental group with 7.5% concentration of *C. odorata* extract on the fifth day of observation, C) Positive control with normal saline on the third day, D) Positive group with normal saline on the fifth day

Table 1. Neutrophils, Macrophages, Lymphocytes count on Histopathological Examination

Group 2										
D-3	Sample (N)	N1	N2	N3	N4	N5	N6	N7	N8	N9
	Neutrophils	7	7	5	6	5	6	8	2	4
	Macrophages	8	19	3	8	6	8	5	3	8
	Lymphocytes	7	24	8	19	9	5	10	7	12
D-5	Sample (N)	N10	N11	N12	N13	N14	N15	N16	N17	N18
	Neutrophils	3	6	5	8	4	6	8	5	7
	Macrophages	2	0	2	0	1	3	6	3	0
	Lymphocytes	9	16	7	2	5	5	5	8	13
Group 1										
D-3	Sample (N)	N1	N2	N3	N4	N5	N6	N7	N8	N9
	Neutrophils	13	18	2	10	12	2	6	11	9
	Macrophages	0	5	1	0	5	4	13	4	0
	Lymphocytes	3	12	2	2	7	5	4	7	3
D-5	Sample (N)	N10	N11	N12	N13	N14	N15	N16	N17	N18
	Neutrophils	15	36	2	7	6	7	25	28	10
	Macrophages	8	7	4	1	3	0	8	3	5
	Lymphocytes	3	10	2	2	5	6	6	3	17

Similar results were found in a study by Braimah et al. (2017) and Charles et al. (2018), who assessed the levels of white blood cells, neutrophils, macrophages, and lymphocytes on the use of *C. odorata* extract in Wistar rats contaminated with Salmonella typhi. There was a decrease in neutrophil levels with an increase in macrophage and lymphocyte levels. The differences in the findings of neutrophil, macrophage and lymphocyte levels in this study are due to differences in the concentration and infiltration process of these three inflammatory cells during the wound healing phase. Neutrophils are the first cells to work in the inflammatory phase (Broughton et al., 2006; Taufik et al., 2024). Neutrophil infiltrate the wound 24-48 hours after wounding, characterized by an increase in neutrophil level, and will decrease four days later. The inflammatory phase is continued by macrophages that migrate from the blood vessels into the wound after the neutrophils have completed their work. The peak concentration of macrophages occurs 48–72 hours after wounding (Isnaini et al., 2019; Ellis et al., 2018). Neutrophils and macrophages play a role in phagocytosis of microorganisms and foreign bodies. The last cells to infiltrate the wound are the lymphocytes in the wound at 72 hours after the wound. Lymphocytes play a role in regulating wound healing by releasing lymphocyte antigens, mediating pathogen clearance by releasing pro-inflammatory mediators, immune regulation, production of extracellular matrix and collagen. The normal wound healing process is characterized by a decrease in the concentration of these three cells in the wound at the end of the inflammatory phase (Rodrigues et al., 2019; Landén et al., 2016; Gonzalez et al., 2016).

The anti-inflammatory effect of *C. odorata* is believed to be due to the flavonoids contained in it such as quercetin and kaempferol which are anti-inflammatory agents, by suppressing nitric oxide, causing vasoconstriction and reducing inflammatory mediators to the target area. Flavonoids contained in *C. odorata* (Quercetin) have anti-inflammatory effects by inhibiting three polymorphonuclear cell functions that play a role in inflammation; lysosomal enzyme release, chemiluminescence response, and superoxide anion production (Isnaini et al., 2023).

Table 2. Mean Count of Neutrophils between Groups

Variable	Mean (cells/mm^3)				P
	G2 H-3	G1 H-3	G2 H-5	G1 H-5	Value
Neutrophils	5.6 ± 1.8	9.2 ± 5.2	5.8 ± 1.7	15.1 ± 11.8	0.014
Macrophages	6.9 ± 1.7	3.7 ± 1.4	1.9 ± 0.7	4.3 ± 0.9	0.035
Lymphocytes	11.2 ± 6.3	5 ± 3.2	7.8 ± 4.4	6 ± 4.8	0.031

Research by Hanh et al. (2011) reported Nitric oxide (NO) has been known to play a role in the regulation of various physiological processes in mammals and excessive production of NO is responsible for the development of inflammatory pathologies. In addition, NF-κB activation plays a role in the inflammatory process and its inhibition is a therapeutic target in inflammatory management. It also reported that fatty acids derived from *C. odorata* are address inhibitors of NO and NF-κB production (Murphy & Evans, 2012).

Conclusion

The histopathological examination revealed that the group treated with *C. odorata* extract exhibited a significant reduction in neutrophils on both the third and fifth days compared to the saline-treated group. Meanwhile, macrophage and lymphocyte levels were higher in the *C. odorata* group on day three, contributing

to the enhanced immune response and wound healing process. By day five, macrophage levels decreased in the *C. odorata* group, indicating a shift in the healing phase. The anti-inflammatory effects of *C. odorata*, attributed to flavonoids like quercetin, suppress the production of nitric oxide and inflammatory mediators, leading to better regulation of immune cell infiltration and more efficient wound healing. This study highlights the potential of *C. odorata* extract as a promising therapeutic agent for treating infected wounds.

Acknowledgements
The authors are grateful to the Institute for Research and Community Service (LPPM) Universitas Syiah Kuala for supporting this study.

Author Contributions
M.A.Y.: Conceptualization, methodology, investigation, writing—original draft preparation; data curation, and software. M.A.: validation, formal analysis. A.D.H.: writing—review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

Funding
This research received no external funding.

Conflicts of Interest
All author declares that there is no conflict of interest.

References

Akiki, R. K., & Mehrzad, R. (2020). Practical Management of Common Skin Injuries, Lacerations, Wounds, Trigger Fingers, and Burns. *The Journal of the American Board of Family Medicine*, *33*(5), 799-808. https://doi.org/10.3122/jabfm.2020.05.200017

Almadani, Y. H., Vorstenbosch, J., Davison, P. G., & Murphy, A. M. (2021, August). Wound Healing: A Comprehensive Review. *Seminars in Plastic Surgery*, *35*(03), 141-144. https://doi.org/10.1055/s-0041-1731791

Aziz, N. A., Mohamad, M., Mohsin, H. F., Hazalin, N. A. M. N., & Hamid, K. A. (2020). The Pharmacological Properties and Medicinal Potential of Chromolaena odorata: A Review. *International Journal of Pharmaceutical, Nutraceutical and Cosmetic Science (IJPNaCS)*, *2*, 30-41. http://dx.doi.org/10.24191/IJPNaCS.v2.04

Becker, N., Hammen, A., Bläsius, F., Weber, C. D., Hildebrand, F., & Horst, K. (2023). Effect of Injury Patterns on the Development of Complications and Trauma-Induced Mortality in Patients Suffering Multiple Trauma. *Journal of Clinical Medicine*, *12*(15), 5111. https://doi.org/10.3390/jcm12155111

Braimah, R. O., Ukpong, D. I., Ndukwe, K. C., & Akinyoola, A. L. (2017). Comparative Study of Anxiety and Depression Following Maxillofacial and Orthopedic Injuries: Study from a Nigerian University Teaching Hospital. *Clinical and Experimental Dental Research*, *3*(6), 215–219. https://doi.org/10.1002/cre2.90

Cañedo-Dorantes, L., & Cañedo-Ayala, M. (2019). Skin Acute Wound Healing: A Comprehensive Review. *International Journal of Inflammation*, *2019*(1), 3706315. https://doi.org/10.1155/2019/3706315

Charles, I. J., & Minakiri, S. I. (2018). Effect of Chromolaena odorata on Hepatotoxicology and Histopathology in the Liver Induced by Salmonella Typhi in Wistar Rats. *Isirima Joshua*, 1-16. http://dx.doi.org/10.19044/esj.2018.v14n12p421

Dai, T., Kharkwal, G. B., Tanaka, M., Huang, Y. Y., Arce, V. J. B. D., & Hamblin, M. R. (2011). Animal Models of External Traumatic Wound Infections. *Virulence*, *2*(4). https://doi.org/10.4161/viru.2.4.16840

Ellis, S., Lin, E. J., & Tartar, D. (2018). Immunology of Wound Healing. *Current Dermatology Reports*, *7*, 350-358. https://doi.org/10.1007/s13671-018-0234-9

Enoch, S., & Leaper, D. J. (2008). Basic Science of Wound Healing. *Surgery*, *26*(2), 37–42. https://doi.org/10.1016/j.mpsur.2007.11.005

Falanga, V., Isseroff, R. R., Soulika, A. M., Romanelli, M., Margolis, D., Kapp, S., ... & Harding, K. (2022). Chronic Wounds. *Nature Reviews Disease Primers*, *8*(1), 50. https://doi.org/10.1038/s41572-022-00377-3

Broughton, I. I., Janis, J. E., & Attinger, C. E. (2006). The Basic Science of Wound Healing. *Plastic and Reconstructive Surgery*, *117*(7S), 12S-34S. https://doi.org/10.1097/01.prs.0000225430.42531.c2

Geraerds, A. J., Haagsma, J. A., Munter, L. D., Kruithof, N., Jongh, M. D., & Polinder, S. (2019). *Medical and Productivity Costs After Trauma*. *PloS One*, *14*(12), e0227131. https://doi.org/10.1371/journal.pone.0227131

Gonzalez, A. C. D. O., Costa, T. F., Andrade, Z. D. A., & Medrado, A. R. A. P. (2016). Wound Healing-A Literature Review. *Anais Brasileiros de Dermatologia*, *91*(5), 614-620. https://doi.org/10.1590/abd1806-4841.20164741

Guo, S. A., & DiPietro, L. A. (2010). Factors Affecting Wound Healing. *Journal of Dental Research*, *89*(3), 219-229. https://doi.org/10.1177/0022034509359125

Gushiken, L. F. S., Beserra, F. P., Bastos, J. K., Jackson, C. J., & Pellizzon, C. H. (2021). Cutaneous Wound Healing: An Update from Physiopathology to

Current Therapies. *Life*, *11*(7), 665. https://doi.org/10.3390/life11070665

Hanh, T. T. T., Hang, D. T. T., Minh, C. V., & Dat, N. T. (2011). Anti-Inflammatory Effects of Fatty Acids Isolated from Chromolaena odorata. *Asian Pacific Journal of Tropical Medicine*, *4*(10), 760–763. https://doi.org/10.1016/S1995-7645(11)60189-2

Hanphanphoom, S., & Krajangsang, S. (2016). Antimicrobial Activity of Chromolaena odorata Extracts Against Bacterial Human Skin Infections. *Modern Applied Science*, *10*(2), 159. http://dx.doi.org/10.5539/mas.v10n2p159

Isnaini, N., Prajaputra, V., & Maryam, S. (2023). Formulation and Evaluation of O/W Body Cream Containing Patchouli Oil (Pogostemon cablin Benth.) and Drumstick Oil (Moringa oleifera) as Potential Moisturizing Agent. *Jurnal Penelitian Pendidikan IPA*, *9*(10), 8001-8007. https://doi.org/10.29303/jppipa.v9i10.4292

Isnaini, N., Songkro, S., Kaewnopparat, N., & Maneenuan, D. (2019). Formulation and Investigation of Antioxidant Potential of O/W Lotions Containing Tamarindus indica L. Fruit Pulp Extract. *Matter: International Journal of Science and Technology*, *5*(2), 100-112. https://dx.doi.org/10.20319/mijst.2019.52.100112

Landén, N. X., Li, D., & Ståhle, M. (2016). Transition from Inflammation to Proliferation: A Critical Step During Wound Healing. *Cellular and Molecular Life Sciences*, *73*, 3861-3885. https://doi.org/10.1007/s00018-016-2268-0

Li, R., Liu, K., Huang, X., Li, D., Ding, J., Liu, B., & Chen, X. (2022). Bioactive Materials Promote Wound Healing Through Modulation of Cell Behaviors. *Advanced Science*, *9*(10), 2105152. https://doi.org/10.1016/j.jtv.2019.09.002

Ling, S. K., Pisar, M. M., & Man, S. (2007). Platelet-Activating Factor (PAF) Receptor Binding Antagonist Activity of the Methanol Extracts and Isolated Flavonoids from Chromolaena odorata (L.) K ING and R OBINSON. *Biological and Pharmaceutical Bulletin*, *30*(6), 1150-1152. https://doi.org/10.1248/bpb.30.1150

Murphy, P. S., & Evans, G. R. (2012). Advances in Wound Healing: A Review of Current Wound Healing Products. *Plastic Surgery International*, *2012*(1), 190436. https://doi.org/10.1155/2012/190436

Dagher, T. N., Al-Bayssari, C., Diene, S. M., Azar, E., & Rolain, J. M. (2020). Bacterial Infection During Wars, Conflicts and Post-Natural Disasters in Asia and the Middle East: A Narrative Review. *Expert Review of Anti-Infective Therapy*, *18*(6), 511-529. https://doi.org/10.1080/14787210.2020.1750952

Olawale, F., Olofinsan, K., & Iwaloye, O. (2022). Biological Activities of Chromolaena odorata: A Mechanistic Review. *South African Journal of Botany*, *144*, 44-57. https://doi.org/10.1016/j.sajb.2021.09.001

Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S., & Saparov, A. (2021). Immunology of Acute and Chronic Wound Healing. *Biomolecules*, *11*(5), 700. https://doi.org/10.3390/biom11050700

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound Healing: A Cellular Perspective. *Physiological Reviews*, *99*(1), 665-706. https://doi.org/10.1152/physrev.00067.2017

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound Healing: A Cellular Perspective. *Physiological Reviews*, *99*(1), 665-706. https://doi.org/10.1152/physrev.00067.2017

Schreml, S., Szeimies, R. M., Prantl, L., Landthaler, M., & Babilas, P. (2010). Wound Healing in the 21st Century. *Journal of the American Academy of Dermatology*, *63*(5), 866-881. https://doi.org/10.1016/j.jaad.2009.10.048

Serra, M. B., Barroso, W. A., Silva, N. N. D., Silva, S. D. N., Borges, A. C. R., Abreu, I. C., & Borges, M. O. D. R. (2017). From Inflammation to Current and Alternative Therapies Involved in Wound Healing. *International Journal of Inflammation*, *2017*(1), 3406215. https://doi.org/10.1155/2017/3406215

Shukla, S. K., Sharma, A. K., Gupta, V., & Yashavarddhan, M. H. (2019). Pharmacological Control of Inflammation in Wound Healing. *Journal of Tissue Viability*, *28*(4), 218-222. https://doi.org/10.1016/j.jtv.2019.09.002

Sorg, H., Tilkorn, D. J., Hager, S., Hauser, J., & Mirastschijski, U. (2017). Skin Wound Healing: An Update on the Current Knowledge and Concepts. *European Surgical Research*, *58*(1-2), 81-94. https://doi.org/10.1159/000454919

Stokes, S. M., Scaife, C. L., Brooke, B. S., Glasgow, R. E., Mulvihill, S. J., Finlayson, S. R., & Jr, T. K. V. (2022). Hospital Costs Following Surgical Complications: A Value-Driven Outcomes Analysis of Cost Savings Due to Complication Prevention. *Annals of Surgery*, *275*(2), e375-e381. https://doi.org/10.1097/SLA.0000000000004243

Taufik, M., Rizal, S., Harnelly, E., Muhammad, S., Syahrizal, D., Prajaputra, V., & Isnaini, N. (2024). The Therapeutic Potential of Aceh Patchouli Oil (Pogostemon cablin Benth.) in Enhancing Full-Thickness Wound Healing in Mice. *Tropical Journal of Natural Product Research*, *8*(1). https://doi.org/10.26538/tjnpr/v8i1.19

Tottoli, E. M., Dorati, R., Genta, I., Chiesa, E., Pisani, S., & Conti, B. (2020). Skin Wound Healing Process and New Emerging Technologies for Skin Wound

Care and Regeneration. *Pharmaceutics*, *12*(8), 735. https://doi.org/10.3390/pharmaceutics12080735

Velnar, T., Bailey, T., & Smrkolj, V. (2009). The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms. *Journal of International Medical Research*, *37*(5), 1528–1542. https://doi.org/10.1177/147323000903700531

Yang, F., Bai, X., Dai, X., & Li, Y. (2021). The Biological Processes During Wound Healing. *Regenerative Medicine*, *16*(4), 373-390. https://doi.org/10.2217/rme-2020-0066

Yuliani, N. S., & Viktor, L. (2015). Pengaruh Ekstrak Daun Chromolaena odorata Terhadap Proses Kesembuhan Luka Insisi pada Tikus Sprague-Dawley. *Jurnal Kajian Veteriner Desember*, *3*(2), 93–99. https://doi.org/10.35508/jkv.v3i2.1034