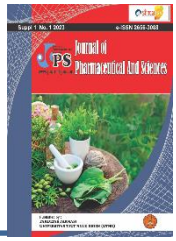
JOURNAL OF PHARMACEUTICAL AND SCIENCES
Electronic ISSN: 2656-3088
Homepage: https://www.journal-jps.com

ORIGINAL ARTICEL

JPS |Suppl 1 | No. 1 | 2023 |pp.91-98



Evaluation of the wound healing activity of ethanol extract fractions from kirinyuh leaves (*Chromolaena odorata* (L.) R.M. King & H. Rob) on incision wounds in white rats (*Rattus norvegicus*)

Evaluasi aktivitas penyembuhan luka fraksi ekstrak etanol daun kirinyuh (*Chromolaena odorata* (L.) R.M. King & H. Rob) pada luka sayat tikus putih (Rattus norvegicus)

M. Rifqi Efendi[1*)], Elisma Elisma[1)], Niken Zahira[1)]

[1)]Department of Pharmacy, Faculty of Medicine and Health Science, Universitas Jambi, Jambi, Indonesia.
**e-mail author: rifqi.efendi09@gmail.com*

ABSTRACT
Kirinyuh (*Chromolaena odorata* (L.) R.M.King & H.Rob) is a plant from the Asteraceae family traditionally used for wound healing. This research aims to evaluate the wound healing activity of n-hexane, ethyl acetate, and n-butanol fractions derived from kirinyuh ethanol extract (*Chromolaena odorata* (L.) R.M.King & H.Rob). The study employed a post-test control group design in vivo using white rats (Rattus norvegicus) induced with a 3 cm long and 2 mm deep incision wound. The test formulations were applied twice a day in the morning and evening for 10 days at a dosage of 0.5 g per treatment. Wound healing effects were assessed based on the percentage of wound healing and histopathology of rat skin. The results indicated that all test fractions demonstrated wound-healing effects. The ethyl acetate fraction exhibited the most significant effect on incision wound healing compared to the positive control and the other two fractions, achieving a 75% wound healing percentage. Additionally, histopathological observations of rat skin revealed improved collagen content and epithelial cell thickness in the ethyl acetate fraction. Therefore, the ethyl acetate fraction holds the potential for development as a topical formulation in wound healing.

***Keywords**: Incision wound, Chromolaena odorata L., Wound healing*

ABSTRAK
Kirinyuh (*Chromolaena odorata* (L.) R.M.King & H.Rob) merupakan tumbuhan dari famili asteraceae yang secara tradisional digunakan sebagai penyembuhan luka. Tujuan dari penelitian ini adalah mengevaluasi aktivitas penyembuhan luka dari fraksi n-heksana, etil asetat, dan n-buthanol dari ekstrak etanol kirinyuh (*Chromolaena odorata* (L.) R.M.King & H.Rob). Penelitian ini dilakukan menggunakan *post test control group design* secara in vivo menggunakan hewan uji tikus putih (*Rattus norvegicus)* yang diinduksi luka sayat dengan panjang luka 3 cm dan kedalaman 2 mm, sediaan uji diaplikasikan 2 kali sehari pada pagi dan sore selama 10 hari sebanyak 0,5 g tiap perlakuan. Efek penyembuhan luka dinilai dari parameter persentase penyembuhan luka dan histopatologi kulit tikus. Hasil penelitian menunjukkan bahwa semua fraksi uji menunjukkan efek penyembuhan luka. Fraksi etil asetat menunjukkan efek penyembuhan luka sayat yang paling baik jika dibandingkan dengan control positif dan dua fraksi lainnya dengan persentase penyembuhan luka 75 %, dan memperlihatkan hasil pengamatan histopatologi kulit tikus dengan jumlah kolagen dan ketebalan sel epitel lang

lebih baik. Oleh karena itu, Fraksi etil asetat memiliki potensi dikembangkan menjadi sediaan topical dalam penyembuhan luka.

***Kata kunci** : Luka Sayat, Chromolaena odorata L., penyembuhan luka*

INTRODUCTION

Incised wounds are a prevalent type of injury in both humans and animals, caused by mechanical trauma like sharp cuts or other cutting objects **(Wilkinson & Hardman, 2020)**. The intricate process of wound healing involves a dynamic interplay among diverse cell types, growth factors, and inflammatory processes aimed at restoring damaged tissues **(Munro & Munro, 2008)**. Despite the array of available treatment methods, there is a growing importance of researching natural substances with wound-healing properties to enhance the healing process and mitigate the risk of complications.

Kirinyuh (Chromolaena odorata (L.) R.M. King & H. Rob) is commonly employed in traditional practices for wound treatment in various communities **(Sirinthipaporn & Jiraungkoorskul, 2017)**. Kirinyuh is recognized for harboring active compounds such as phenolic acids (protocatechuic, P-hydroxybenzoic, P-coumaric, ferulic, and vanillic acids) and a complex blend of lipophilic flavonoid aglycones (flavanones, flavonol, flavones, and chalcones). These compounds play a crucial role as primary antioxidants, safeguarding skin cells against oxidative damage (Phan et al., 2001). Despite its widespread usage, there is a paucity of scientific research substantiating the efficacy of this plant in wound healing.

Hence, this study aims to assess the efficacy of ethanol leaf extract fractions of Kirinyuh in the healing of incised wounds in white rats (Rattus norvegicus). Ethanol extract fractions were chosen for their ability to concentrate active compounds from Kirinyuh leaves with potential wound-healing properties. The selection of white rats is based on the similarities in their wound-healing system to humans and the availability of standardized white rat models (Dorsett-Martin, 2004).

This research endeavors to provide compelling scientific evidence supporting the use of Kirinyuh leaves as a natural remedy for wound healing. The anticipated results are poised to offer novel insights for the development of more effective and sustainable wound-healing therapies. Additionally, this study aims to deepen our understanding of the mechanisms of action of ethanol leaf extract fractions of Kirinyuh in the wound healing process.

RESEARCH METHODOLOGY

Equipment

The equipment utilized in this study includes test tubes, test tube racks, porcelain dishes, crucible dishes, surgical knives, calipers, cotton, maceration containers (dark bottles), rotary evaporator (IKA RV 8 V®), measuring glasses, analytical balances (Kern®), glass funnels, filter paper, Erlenmeyer flasks, beakers, stirring rods, grinder, hot plate, pipettes, oven, separating funnel, forceps, animal cages, drinking vessels, and animal hair shavers.

Materials

The materials used consist of Chromolaena odorata L. leaf extract, 70% ethanol distillate, n-hexane (Brathacho®), ethyl acetate (Brathacho®), butanol (Brathacho®), acepromazine (Castran®), Vaselin flavum (Brathacho®), distilled water (aquadest), 10% Povidone iodine ointment (Betadine®), Veet® Cream, Mg powder, concentrated HCl, FeCl3 1%, Mayer's reagent, chloroform ammonia, 2 N sulfuric acid (H2SO4), anhydrous acetic acid, and test animal feed.

Sample Collection and Identification

In this research, the sample used was Chromolaena odorata L. leaves collected from Siulak District, Kerinci Regency, Jambi Province. Sample collection was conducted in the morning by cutting fresh leaves at the plant's apex, totaling 5 kg. Subsequently, a determination process was carried out to identify the research sample, performed at the Herbarium of Universitas Padjajaran with reference number 12/HB/12/2022.

Extraction and Fractionation of Chromolaena odorata (L.) Leaves

The extraction of Kirinyuh leaves was conducted using the maceration method with 70% ethanol as the solvent. Maceration was carried out in brown bottles with a sample-to-solvent ratio of 1:10. The maceration process was repeated three times. The maceration result was concentrated using a rotary evaporator, yielding a concentrated ethanol extract of Kirinyuh leaves (Kementerian Kesehatan RI, 2017). The ethanol extract of Kirinyuh leaves underwent fractionalization in stages using n-hexane, ethyl acetate, and n-butanol as solvents. Subsequently, each resulting fraction was further concentrated using a rotary evaporator until a viscosity consistency was achieved (Efendi, 2019).

Characteristics of the Extract

The examination of the ethanol extract from Kirinyuh leaves encompasses specific parameters, including identity and organoleptic tests, followed by non-specific parameters involving tests for water content and ash content (Kementerian Kesehatan RI, 2017). Phytochemical screening was conducted on the extract, which includes tests for flavonoids, alkaloids, saponins, steroids, terpenoids, and phenols (Efendi, 2019; Efendi, Gusti, Syahrial, & Rusdi, 2023).

Preparation of Kirinyuh Leaf Fraction Formulation

Each formula of Kirinyuh leaf ethanol extract fraction was prepared with a concentration of 10%, using vaselin flavum as the base. The n-hexane fraction (P1), ethyl acetate fraction (P2), and n-butanol fraction (P3) were included. The positive control used was Betadine ointment, while the negative control used was vaselin flavum.

Animal Treatment

This study utilized 25 male rats weighing 200-300 g and aged 2-3 months. The animals were subjected to treatment using a completely randomized design (CRD) with a Post-test Only Control Group Design. The rats were divided into five treatment groups (K+, K-, P1, P2, P3), each consisting of 5 male white rats. They were housed in an environmentally controlled room with access to food and water ad libitum. Animal procedures strictly adhered to institutional protocols and followed the animal care regulations outlined by the Ethics Committee of the Faculty of Medicine, Universitas Andalas (No. 1095/UN.16.2/KEP-FK/2023).

Incised Wound Creation

White rats, acclimated for 7 days, were weighed before each experimental group. The fur on the rats' backs, at the intended incision site, was shaved using a razor or other shaving tool. Rats were anesthetized with acepromazine (Castran®) through intramuscular injection at a dose of 0.2 mg/kgBW. Subsequently, an incision was made on the rat's back, measuring 3 cm in length and 2 mm in depth, using a surgical scalpel, with the incision depth marked at 2 mm. The test preparations were applied to the wounds twice a day, in the morning and evening, for 10 days, at a dose of 0.5 g per treatment.

Observation of Incised Wound Healing

Measurement of Wound Length

The measurement of incised wound length was initiated on the first day of treatment and subsequently recorded once a day for 10 days, using calipers for macroscopic assessment. To determine the percentage of wound healing, the following formula can be employed: (Sanik et al., 2022).

$$P\% = \frac{d0-dx}{d0} \times 100\%$$

Note:
P%: Percentage of Wound Healing
d0: Wound Length on the first day
dx: Wound Length on day x

Histopathological Observation

Wounds were created on the skin of rats through incisions, and samples were collected to prepare microscopic slides using the paraffin method and stained with Hematoxylin and Eosin (HE). Cutting was performed using a rotary microtome and disposable blades. Paraffin ribbons were collected and arranged at 60°C, then stained with hematoxylin and eosin. Microscopic images were evaluated in five fields of view using a light microscope with a magnification of 400x. The assessment was based on observations of cell infiltration, collagen formation, and the condition of the epithelium (Kurniawaty et al., 2022; Wilkinson and Hardman, 2020).

Data Analysis

The secondary metabolites in the fractions of Kirinyuh leaf extract were described descriptively.

Statistical analysis of wound healing parameters using SPSS involves testing for normality and homogeneity of data. If the data was normally distributed and homogenous, the One-Way ANOVA test determined the average differences among groups, followed by the Duncan test to detect significant differences among treatments.

RESULTS AND DISCUSSION

In this study, we utilized samples of Kirinyuh leaf ethanol extract that were fractionated using three different solvents: n-hexane, ethyl acetate, and n-butanol, each with varying polarity levels. Each fraction was prepared with a test concentration of 10%, and vaseline flavum was employed as the carrier. The activities of the three fractions of Kirinyuh leaf ethanol extract were compared with Betadine ointment and Vaseline flavum.

Characterization of the Kirinyuh leaf ethanol extract was conducted based on specific and non-specific parameters. The determination of specific parameters aimed to provide the identity of the extract, such as the nomenclature description, extract name, organoleptic features, regional plant names, plant parts, and plant names. Organoleptic determination of the extract aimed to describe its form, color, aroma, and taste (Sirinthipaporn & Jiraungkoorskul, 2017). The test results can be found in Table 1.

Table 1. Test Results of Specific Parameters for Ethanol Extract of Kirinyuh Leaves

Specific Parameters		Results
Organoleptic	Form	Viscous Liquid
	Odor	Specific Odor
	Color	Dark Green Color
	Taste	Bitter
Identity	Extract Name	*Choromolaena odorata* L. Extractum
	Species of the plant	*Choromolaena odorata* L.
	Plant part used	Daun
	Indonesian name	Kirinyuh
	Family	Asteraceae

The test conducted is a non-specific test, which includes ash content and water content. The obtained results can be observed in Table 2.

Table 2. Results of Non-Specific Parameter Testing for Ethanol Extract of Kirinyuh Leaves

Non-Specific Parameters	Results
Ash Content	10,3%
Water Content	15,3%

Measurement of ash content aims to provide an overview of the internal and external mineral content in the initial stages until the formation of the concentrated extract. The examination of ash content is carried out by heating the substance at a high temperature, where organic compounds and their derivatives decompose and evaporate, leaving only mineral and inorganic elements. Determination of water content is conducted using gravimetric methods, aiming to ascertain the extent of water content, which is related to the purity level and the potential for possible contamination (Kementerian Kesehatan RI, 2017; Sirinthipaporn & Jiraungkoorskul, 2017).

Phytochemical screening is conducted to identify the content of secondary metabolite compounds in a natural substance. The results of the phytochemical screening can be seen in Table 3.

Phytochemical screening of Kirinyuh leaf extract indicates the presence of flavonoids, alkaloids, saponins, steroids, and phenols. These results align with previous research findings that also involved phytochemical screening of Kirinyuh leaf extract conducted by (Phan et al., 2001) and (Sirinthipaporn & Jiraungkoorskul, 2017).

Table 3. Results of Phytochemical Screening forEthanol Extract of Kirinyuh Leaves.

Secondary Metabolite	Results
Flavonoid	+
Alkaloid	+
Saponin	+
Steroid	+
Phenol	+

In the testing of the ethanol leaf extract fractions of Kirinyuh, the experiment involved inducing incised wounds on the backs of rats. The type of wound given corresponds to a stage 2 wound, where the color of the wound appears red and involves the epidermis and dermis layers. Each group received different treatments, namely the application of Vaseline flavum in the negative control group (K-), the use of Betadine ointment in the positive control group (K+), n-hexane fraction (Treatment P1), ethyl acetate fraction (Treatment P2), and n-butanol fraction (Treatment P3), each formulated with Vaseline flavum at a concentration of 10%.

The results of the study on wound healing are presented in Table 4 and Figure 1. The ethyl acetate fraction was found to have the most effective wound-healing effect compared to other treatments. It had an average wound length of 0.838 cm and a healing percentage of 73%. The average wound length in the negative control group was not significantly different from the positive control and treatment P1 and P2, although their healing percentages differed. This suggests that wounds can heal naturally through the normal stages of cell regeneration. However, Treatment P2 showed significantly different results from the other four test groups, indicating that it has the potential to accelerate the wound healing process.

Table 4. Average Wound Length and Percentage of Wound Healing

Treatment Groups	Average Wound Length ± SD
K-	1.404 ± 0.036^{b}
K+	1.312 ± 0.132^{b}
P1	1.284 ± 0.063^{b}
P2	0.838 ± 0.512^{a}
P3	1.198 ± 0.1^{b}

Note :

1. Small lowercase superscripts on the same line indicate significant differences (P<0,05).
2. K- : control negative (Vaseline flavum); K+ : control Positive (Betadine salep); P1 : Treatment 1 (Fraction n-hexane 10%); P2 : Treatment 2 (Fraction ethyl acetate 10%); P3 : Treatment 3 (Fraction n-butanol 10%).

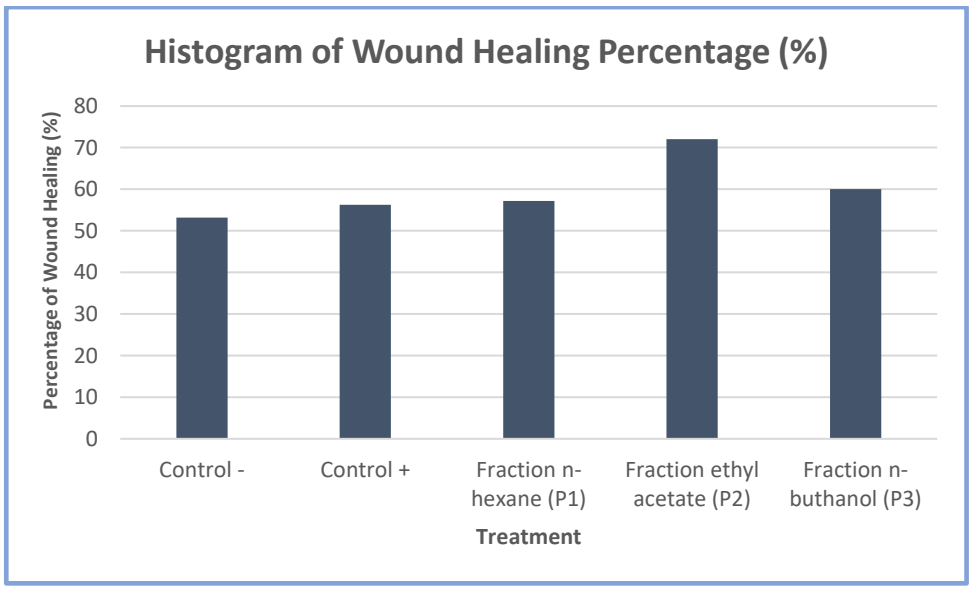


Figure 1. Histogram of Wound Healing Percentage

Histology observations were conducted to assess and observe the level of wound healing in rat skin. The variables examined included collagen production and epithelial thickness. According to the theory of the healing process, collagen production is expected to increase because it plays a crucial role in repairing damaged or lost tissue. Fibroblasts undergo a phenotypic change into myofibroblasts, which play a role in wound contraction. The faster the re-epithelialization process occurs, the sooner the wound closes, accelerating overall wound healing (Kondo, 2007; Monavarian et al., 2019; Wilkinson and Hardman, 2020).

Based on microscopic observations in Figure 2, the density of collagen formation and the thickness of the epithelium for each treatment can be seen. Treatment P2 (ethyl acetate fraction) shows the most optimal collagen density and epithelial thickness compared to the positive control, followed by Treatment P3 (butanol fraction) and P1 (n-hexane fraction). However, histopathological observations prove that although the average wound length and percentage of wound healing from the negative control, positive control, Treatment 1, and Treatment 3 are within one subset, each treatment does not show identical growth of collagen and epithelial cells.

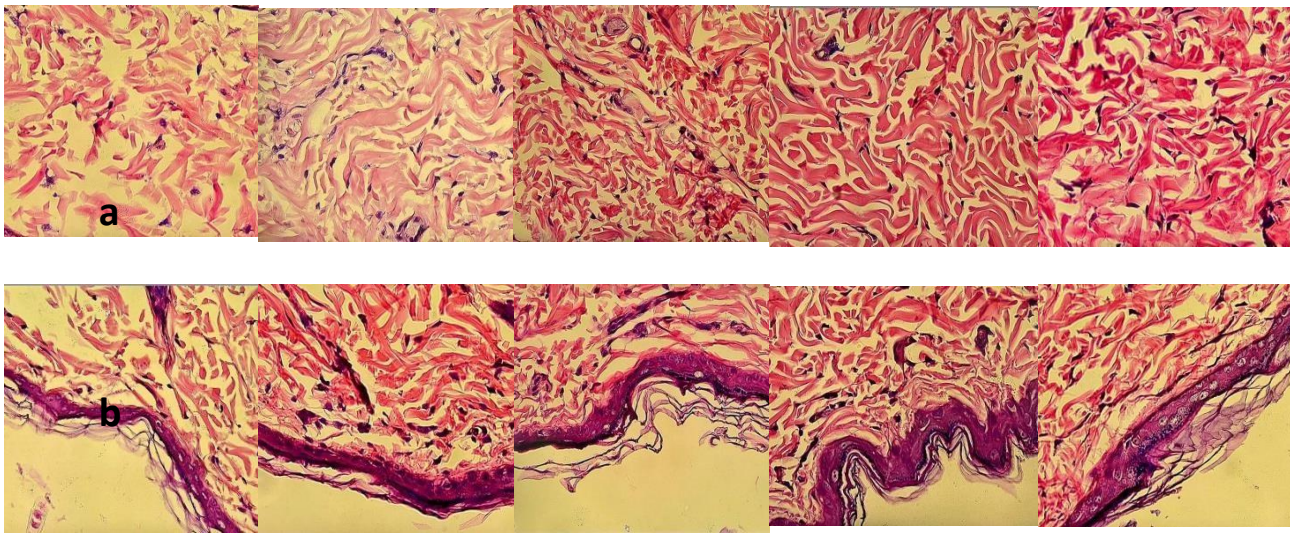


Figure 2. Histopathology of Rat Skin (a. Collagen, b. Epithelial Cells).

In the wound healing process, there are certain compounds believed to play a role in reducing the length of the wound on the rat's back every day, accelerating the closure process, and enhancing healing. These compounds can be found in Kirinyuh leaf extract, including flavonoids, saponins, phenols, and alkaloids. Analysis of dichloromethane (semipolar) extract from Kirinyuh leaves identified several flavonoid compounds such as 2'-hydroxy-3,4,4',5',6'-pentamethoxy-chalcone, 2',4-dihydroxy-4',5',6'-trimethoxy-chalcone, scutellarein tetramethyl ether, Sinensetin, and 2'-hydroxy-4,4',5'.6'-tetramethoxychalcone (Barua, Sharma, Thyagarajan, & Hertz, 1978).

Flavonoids work by inhibiting the formation of prostaglandins and other inflammatory mediators, such as leukotrienes. The reduction in the production of prostaglandins and leukotrienes as inflammatory mediators can accelerate the transition from inflammation to the proliferation process, supporting faster wound healing. Saponins and phenols, also found in Kirinyuh leaf extract, enhance the proliferation process of monocytes which can affect the number of macrophages. An increased number of macrophages around the wound area can enhance the secretion of growth factors that play a role in proliferation, increase fibroblast migration, and increase the amount of

synthesized collagen, all contributing to a rapid proliferation process (Carvalho et al., 2021).

These findings align with previous research indicating that Kirinyuh leaf extract supports the hemostasis and wound healing processes (Pandith et al., 2013). Other studies (Phan et al., 2001) also support these findings by stating that the phenolic compounds present in Kirinyuh plants have strong antioxidant activity and support wound healing. The flavonoid compounds in Kirinyuh leaves are also believed to have the potential to accelerate wound healing, regulate inflammatory cytokines, reduce the number of mononuclear cells in the proliferation phase, accelerate the rate of wound contraction, and promote vasculogenesis and angiogenesis, as found in research by (Carvalho et al., 2021).

CONCLUSION

The three fractions tested from the ethanol extract of Kirinyuh leaves show potential as wound healing agents, especially when compared to the positive control. Among these fractions, the ethyl acetate fraction is the most effective in accelerating the wound-healing process. This fraction demonstrated an average wound length of 0.838 cm and a healing percentage of 73%. Therefore, the ethyl acetate fraction has the potential to be developed as a topical formulation in the context of wound healing.

REFERENCES

Barua, R. N., Sharma, R. P., Thyagarajan, G., & Hertz, W. (1978). Flavonoids of Chromolaena odorata. *Phytochemistry*, *17*(10), 1807–1808. https://doi.org/10.1016/S0031-9422(00)88710-5

Carvalho, M. T. B., Araújo-Filho, H. G., Barreto, A. S., Quintans-Júnior, L. J., Quintans, J. S. S., & Barreto, R. S. S. (2021). Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *90*. https://doi.org/10.1016/J.PHYMED.2021.153636

Dorsett-Martin, W. A. (2004). Rat models of skin wound healing: a review. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *12*(6), 591–599. https://doi.org/10.1111/J.1067-1927.2004.12601.X

Efendi, M. R. (2019). Skrining Aktivitas Antibakteri Fraksi Kelopak Bunga Mussaenda frondosa L. *Journal of Pharmaceutical And Sciences*, *2*(1), 38–44. https://doi.org/10.36490/JOURNAL-JPS.COM.V2I1.18

Efendi, M. R., Gusti, D. R., Syahrial, A. D., & Rusdi, M. S. (2023). Evaluation of Coffee Mistletoe Leaf Ethanol Extract on Burn Healing Activity on White Male Rat. *Journal of Pharmaceutical and Sciences*, *6*(3), 1059–1067. https://doi.org/10.36490/JOURNAL-JPS.COM.V6I3.72

Kementerian Kesehatan RI. (2017). *Farmakope Herbal Indonesia* (2nd ed.). Jakarta: Direktorat Jenderal Kefarmasian dan Alat Kesehatan. Retrieved from https://farmalkes.kemkes.go.id/2020/08/farmakope-herbal-indonesia-edisi-ii-tahun-2017-3/

Kurniawaty, E., Marga, M. P., Kurniati, I., A.Audah, K., & Andriani, S. (2022). The Effect of Mangrove Leaf Extract (Bruguiera Gymnorrhiza) on the Histopathology of the Skin of White Rats (Rattus Norvegicus) Which Has Been Subjected. *Current Science*, *2*(2), 94–101. Retrieved from https://currentscience.info/index.php/cs/article/view/59

Munro, R., & Munro, H. M. C. (2008). Wounds and Injuries. *Animal Abuse and Unlawful Killing*, 30–47. https://doi.org/10.1016/B978-0-7020-2878-6.50015-5

Pandith, H., Zhang, X., Liggett, J., Min, K.-W., Gritsanapan, W., & Baek, S. J. (2013). Hemostatic and Wound Healing Properties of Chromolaena odorata Leaf Extract. *ISRN Dermatology*, *2013*, 1–8. https://doi.org/10.1155/2013/168269

Phan, T. T., Wang, L., See, P., Grayer, R. J., Chan, S. Y., & Lee, S. T. (2001). Phenolic compounds of Chromolaena odorata protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. *Biological & Pharmaceutical Bulletin*, *24*(12), 1373–1379. https://doi.org/10.1248/BPB.24.1373

Sanik, F., Rahman, H., Rahman, A. O., Samudra, A. G., Floris, C. de, & Muhaimin, M. (2022). Incision wound healing test ethanolic extract gel from Ekor Naga (Rhaphidophora pinnata

(L.f) Schott) leaves in white male rats. *Pharmaciana*, *12*(2), 173–180. https://doi.org/10.12928/PHARMACIANA.V12I2.22825

Sirinthipaporn, A., & Jiraungkoorskul, W. (2017). Wound Healing Property Review of Siam Weed, Chromolaena odorata. *Pharmacognosy Reviews*, *11*(21), 35–38. https://doi.org/10.4103/PHREV.PHREV_53_16

Wilkinson, H. N., & Hardman, M. J. (2020). Wound healing: cellular mechanisms and pathological outcomes. *Open Biology*, *10*(9), 341–370. https://doi.org/10.1098/RSOB.200223