Majalah Obat Tradisional (Trad. Med. J.), September-December 2024
Vol. 29(3), p 357-365
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 15-06-2024
Revised : 28-07-2024
Accepted : 04-11-2024

In vivo Wound Healing Activity of Cold and Hot-Pressed Methods of Tamanu Oil (*Calophyllum inophyllum* L.) and Their Fatty Acid Composition

Rita Rakhmawati[1]*, Dinar Sari Cahyaningrum Wahyuni[1], Ahmad Ainurofiq[1], Saptono Hadi[1], Syaiful Choiri[1], Estu Retnaningtyas[1], Tiara Dewi Salindri Pratama[1]
[1] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia

ABSTRACT

Tamanu oil is a promising natural wound healer due to its chemical compounds, particularly fatty acids. Cold-pressed or hot-pressed methods are commonly used to obtain this oil from Calophyllum seeds. However, there needs to be research documenting the impact of these two extraction methods on the fatty acid profile and wound healing activity. Therefore, this study aims to characterize the fatty acid profiles of cold-pressed and hot-pressed tamanu oils using gas chromatography (GC) and evaluate their wound healing activity *in vivo*. The fatty acid profiles were analyzed using GC, and the wound healing tests were conducted on animal subjects divided into four groups: negative control, positive control (Bioplacenton®), cold-pressed tamanu oil, and hot-pressed tamanu oil. Cold-pressed tamanu oil showed superior fatty acid characteristics with an acid value of 38,71 Mg KOH/g fat and a peroxide value of 3,0095 mEq O2/kg, indicating that oil is stable against oxidation. The length of the wound was observed daily for up to 8 days to assess its effect. The parameters observed were the percentage of wound healing and the total area under curve (AUC) based on the average length of the wound. Cold-pressed tamanu oil demonstrated the highest wound healing efficacy compared to both the positive control and hot-pressed tamanu oils. Both cold (11,67± 0,78) and hot-pressed tamanu oil (11,87 ± 0,61) exhibited significant differences in AUC value compared to the negative control group (13,07 ± 0,38), highlighting the potential of tamanu oil as a wound healing agent.
Keywords: *Calophyllum inophyllum*; Fatty acid; in vivo; Nyamplung; Tamanu oil; Wound healing

INTRODUCTION

The primary function of the skin is to act as a protective barrier against physical damage, water loss, and the invasion of toxic agents. When the normal anatomical structure of the skin is damaged, it results in a wound, and the process of healing aims to restore this structure and its functions (Feng et al., 2024). Wound healing involves several phases, such as hemostasis, inflammation, and proliferation (Choudhary et al., 2024). This process is typically well-organized and relies on the coordinated efforts of platelets, keratinocytes, immune cells, microvascular cells, and fibroblasts to repair tissue and restore its integrity (Qiu et al., 2024).

Continued research and development in wound healing focus on identifying and employing both synthetic and biological materials that enhance wound management. The goal is to find materials that not only expedite healing but also reduce the risk of infection and improve clinical outcomes for patients suffering from both acute and chronic wounds (Maleki et al., 2024; Sanpinit et al., 2024).

***Corresponding author : Rita Rakhmawati**
Email : ritarakhmawati@staff.uns.ac.id

Over the years, natural products have become the preferred choice for wound healing and skin care in many countries. The consistency of their use has proven effective in addressing various skin issues such as acne, eczema, and dermatitis (Fan et al., 2024; Saeidi et al., 2024; Zhao et al., 2024). Besides, support from scientific research further confirms the significant benefits of these natural ingredients in maintaining skin health and speeding up wound healing (Gunawan et al., 2021; Krishnappa et al., 2024; Pathak & Gadgoli, 2024). In addition, natural products remain popular due to their focus on safety, effectiveness, and affordability.

Tamanu oil or Nyamplung (in Javanese) oil is a promising natural product as a wound healing agent. Tamanu seeds (*Calophyllum inophyllum* L.) can be processed using various extraction methods such as pressing. The cold-pressed method involves pressing dry seeds directly using a screw system, while the hot-pressed method involves steaming Tamanu seeds first to remove the resin before being pressed, usually for the production of biofuel (Nachippan et al., 2022; Nguyen et al., 2021). Empirically, this oil is used to treat skin diseases. Tamanu oil is effective as an anti-inflammatory agent for treating skin wounds (Ansel et al., 2016; Nguyen et al., 2017). Phytochemical screening of tamanu oil report by Hasibuan et al. (2013) reveals that tamanu contains steroids, flavonoids, saponins, and triterpenoids, which contribute to wound healing. Saponins in the oil can stimulate collagen formation, thereby aiding wound healing (Morikawa et al., 2018). According to Budiawan et al. (2023), flavonoids and saponins can also help normalize the inflammatory phase with antimicrobials that help fight infections in the wound area. Flavonoids can reduce ROS lipid peroxidation, enhancing collagen growth and accelerating the disappearance of erythema. Furthermore, tamanu oil contains unsaturated fatty acids like oleic acid (36.57%) and linoleic acid (26.33%), which play a role in speeding up wound healing (Agustin et al., 2016).

Calophyllolide, the main compound in tamanu oil, is reported to have anti-inflammatory, antimicrobial, and anticoagulant activities (Gunawan et al., 2020; Liu et al., 2015). The finding aligns with research conducted by Yimdjo et al. (2004), which reported that calophyllolide has antimicrobial activity against *S. aureus* with an inhibition zone of 16 mm. The anti-inflammatory activity of calophyllolide is supported by Nguyen et al. (2017), who reported that by day seven post-treatment, wound closure reached 80%, and by day 14, it reached 97%. However, there have been no reports on the fatty acid profile of tamanu oil and its activity in wound healing.

This study aims to characterize the fatty acid profile of two types of tamanu oil using GC and determine whether tamanu oil obtained from two different extraction methods has the same activity in wound healing *in vivo*. The fatty acid profile of pure tamanu oil is analyzed using GC.

MATERIALS AND METHODS

Materials

The experimental animals used were male rats of the Wistar strain aged 2-3 months with a body weight of 150-200 grams. Approval was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital, Number 1.690/IX/HREC/2023. The materials were cold-pressed and hot-pressed tamanu oil (CV. Plantanesia), commercial product® (Kimia Farma) gel, *scalpel* (B-Braun), and *alcohol swabs*.

Methods

The study is an experimental research. The fatty acid profile of tamanu oil was characterized using GC. For the wound healing test, the animal subjects were divided into four groups according to the Frederer formula, i.e., negative control (without treatment), positive control (commercial product®), cold-pressed tamanu oil, and hot-pressed tamanu oil. The rats were placed in a sealed cage, so each rat was placed in one cage. Before treatment, rats were acclimatized for seven days to adapt to the new environment. The rats were shaved on their backs and then cleaned with an alcohol swab. After that, an incision wound was made along 2 cm with a depth of 2 mm using scalpel number 11 to produce a stage 2 acute wound, which is a wound on the dermis tissue but it does not damage muscle tissue (Alaudiin et al., 2016). The tamanu oil was applied evenly on the injured skin two times a day. Observation of the length of the wound was done every day to see its effect for up to 8 days of observation. The parameters observed were the percentage of wound healing and the total AUC based on the average length of the wound. The percentage of wound healing was obtained by measuring the average length of the wound for 8 days of observation. The percentage of wound healing was calculated based on this formula:

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

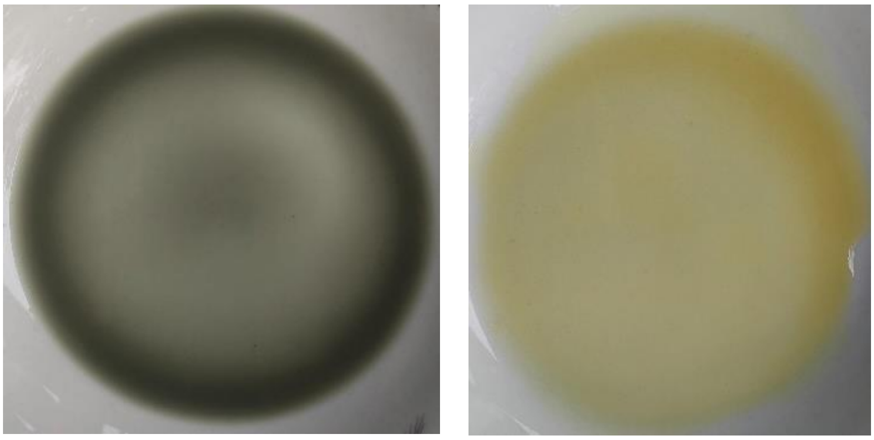
Information: P%: The percentage of wound healing; do: The length of the initial wound; dx: The length of the wound on the day of observation

In addition, the total AUC of the wound length was calculated. The data obtained were then statistically analyzed using One-Way ANOVA and post-hoc LSD to see whether cold-pressed and hot-pressed tamanu oil have a wound-healing effect.

RESULTS

The fatty acid profiles of cold-pressed and hot-pressed tamanu oil using gas chromatography

The tamanu oil derived from both hot-pressed and cold-pressed methods is visually represented in Figure 1. The cold-pressed tamanu oil exhibits a brighter color compared to the darker hue of the hot-pressed oil. The fatty acid profiles of the oils obtained from these methods are detailed in Table I. Analysis revealed that cold-pressed oil contained higher percentages of oleic acid, linoleic acid, and stearic acid. In contrast, palmitic acid was more abundant in the hot-pressed oil by a difference of 1.28%. Furthermore, cold-pressed oil demonstrated lower acid and peroxide values compared to hot-pressed oil, indicating that oil is more stable against oxidation.



(a) (b)

Figure 1. The appearance of tamanu oil obtained by hot pressing (a) and cold pressing (b)

Table I. Comparison of the characteristics and fatty acid profiles of tamanu oil obtained through hot pressing and cold pressing

No.	Parameters	Unit	Hot pressed	Cold pressed	Method
1	Colors	-	Blackish-green	Yellowish-green	SNI 01-2891-1992 point 1.2
2	Linoleic acid	%	22,16	25,69	18-6-1/MU/SMM-SIG (GC)
3	Oleic acid	%	38,93	42,65	18-6-1/MU/SMM-SIG (GC)
4	Stearic acid	%	15,90	16,50	18-6-1/MU/SMM-SIG (GC)
5	Palmitic acid	%	14,21	12,93	18-6-1/MU/SMM-SIG (GC)
6	Acid value	Mg KOH/g fat	45,80	38,71	18-11-17/MU/SMM-SIG (Titrimetry)
7	Peroxide value	mEq O2/kg	4,5005	3,0095	AOAC Official method 965.33

Table II. Percentage of wound healing (%)

Days	Wound Healing Percentage (%) ± SD			
	Negative Control	Positive Control	Cold-Pressed Tamanu Oil	Hot-Pressed Tamanu Oil
0	0,00 ± 0,000	0,00 ± 0,000	0,00 ± 0,000	0,00 ± 0,000
1	5,58 ± 1,934	8,00 ± 2,000	5,67 ± 1,571	6,42 ± 2,035
2	11,54 ± 2,830	16,33 ± 3,933	11,08 ± 2,888	12,25 ± 3,205
3	15,25 ± 2,465	19,88 ± 4,347	17,29 ± 4,729	17,92 ± 4,652
4	18,92 ± 2,131	23,83 ± 4,956	23,29 ± 6,558	23,92 ± 4,466
5	22,08 ± 2,893	30,33 ± 4,779	33,29 ± 6,238	31,25 ± 5,663
6	25,46 ± 5,662	36,46 ± 5,728	45,04 ± 8,149	38,75 ± 6,656
7	29,71 ± 5,192	45,54 ± 5,207	50,62 ± 8,051	47,67 ± 8,329
8	35,63 ± 4,134	57,25 ± 5,392	61,17 ± 8,965	57,33 ± 9,092

The wound-healing activity of cold-pressed and hot-pressed tamanu oil

The measurement results of the average percentage of wound healing (Table II) showed that cold-pressed tamanu oil had the highest wound healing activity on day 8 compared to other groups. The shorter the wound, the more the percentage of wound healing increased and the smaller the total AUC. The average percentage of wound healing in each group increased every day as shown in Figure 2. All of the groups were estimated to have an inflammatory phase on days 1 through 3 which was marked by redness and swelling around the wound. The wound healing phase in this study was estimated to only occur until the proliferative phase. This phase takes place from the 3rd day until the 14th day when the wound has been cleansed from the tissue.

The increase in the percentage of wound healing occurred in all groups. Wound healing is the body's natural process for overcoming tissue damage so normal and negative control also

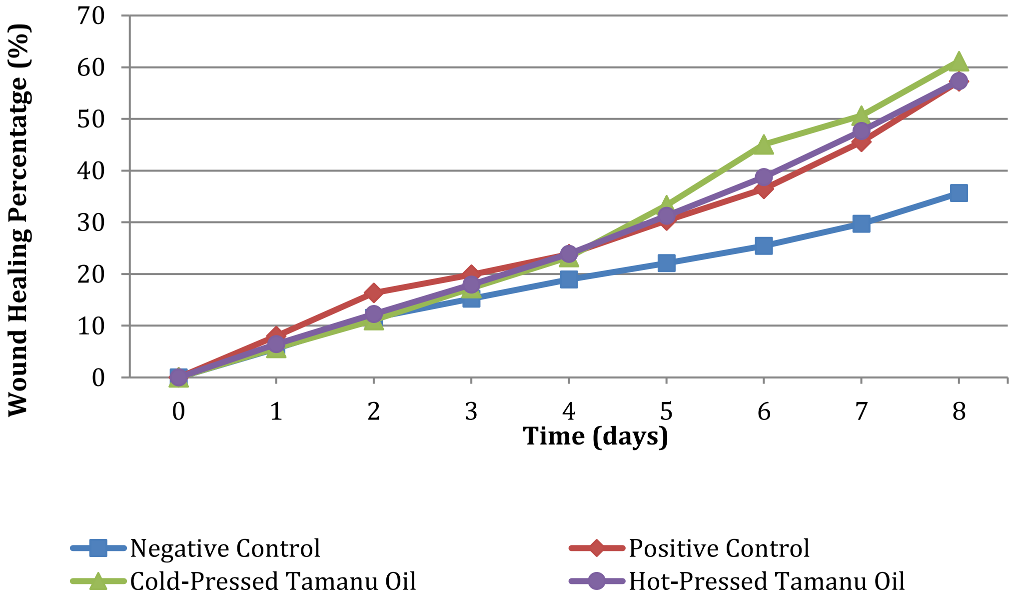


Figure 2. Measurement of wound healing percentage for each treatment group during an 8-day observation period

increases the percentage of wound healing. The lowest percentage of wound healing was in negative control. This was because the group did not contain compounds that were effective as a wound cover. The percentage of wound healing in positive control and the two groups of tamanu oil had almost the same value.

The ability of the tamanu oil as a wound cover was also seen based on the total AUC of the wound length presented in Table III. The average total AUC of the wound length on the cold-pressed tamanu oil had the lowest value compared to the other groups. Based on the One-Way ANOVA analysis on the percentage of wound healing and total AUC, there were significant differences between groups (p-value <0.05). Post hoc LSD analysis showed a significant difference (p-value <0.05) in negative control with the cold-pressed tamanu oil, hot-pressed tamanu oil, and with the positive control. This proved that the tamanu oil both cold-pressed and hot-pressed had an activity as a wound closure compared to negative control with cold-pressed had slightly higher wound healing activity.

DISCUSSIONS

Wound healing is a multifaceted process requiring the coordinated efforts of different tissues and cell lineages to eliminate foreign bodies and inflammation, generate granulation tissue, mature scars, and restore lost or damaged cells and tissue layers (He et al., 2023). The wound healing process comprises four consecutive and coordinated phases: hemostasis, inflammation, proliferation, and remodeling. Each occurs in a specific order to ensure effective healing. Hemostasis initiates immediately after tissue damage, activating platelets to form blood clots and a platelet plug, while growth factors commence tissue repair. Prostaglandin H2 is converted into thromboxane A2 (TXA2), which activates platelets, constricts blood vessels, and triggers the release of macrophages, neutrophils, and endothelial cells. Following hemostasis, the inflammatory phase recruits immune cells like leukocytes and neutrophils to eliminate pathogens and debris, with monocytes differentiating into macrophages to intensify the immune response. These cells release cytokines and growth factors, stimulating fibroblasts and epithelial cells for the subsequent phase. The proliferation phase, lasting from days to weeks, reduces damaged tissue through processes like epidermal regeneration, angiogenesis, granulation tissue formation, and collagen deposition. Activated fibroblasts synthesize extracellular matrix proteins and collagen, forming granulation tissue as a foundation for new extracellular matrix and blood vessels. Cytokines facilitate re-epithelialization, where epithelial cells multiply to form a new epidermal barrier, while fibroblasts differentiate into myofibroblasts, inducing wound contraction. The final remodeling phase, which can extend up to 2 years, transforms the extracellular matrix into a mature scar, substituting collagen type III to type I, reducing blood vessels, and forming mature

Table III. Total AUC of the wound length

Groups	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average ± SD
Negative Control	13,37	12,76	12,86	13,42	12,59	13,44	13,07 ± 0,38 [b]
Positive Control	11,00	12,36	12,17	11,82	12,38	11,21	11,82 ± 0,59 [a]
Cold-pressed TO	10.93	11.06	12.48	11.19	11.56	12.80	11,67 ± 0,78 [a]
Hot-pressed TO	11.64	12.15	12.23	11.43	11.05	12.73	11,87 ± 0,61 [a]

a: Significantly different from negative controls (p-value> 0.05); b: Significantly different from positive control (p-value> 0.05)

avascular tissue, ultimately restoring up to 80% of the skin's original tensile strength (Criollo-Mendoza et al., 2023).

Plant-based products have been used as wound-healing agents for a long time. Extensive research has confirmed their benefits across various aspects, spanning from *in vitro* to clinical trials (Ghanadian et al., 2022; Pelin et al., 2023). In the context of wound healing, these products have consistently shown effectiveness and remain a focal point in modern medical research. Tamanu or *Calophyllum inophyllum* is known as a traditional medicine that provides broad health benefits, especially for skin care. Almost every part of this plant has beneficial properties. The resin extracted from its stem is used to treat bacterial skin infections. At the same time, the oil from this plant is beneficial in treating various skin conditions such as psoriasis, scars, acne, burns, herpes, and dermatitis (Léguillier et al., 2015; Hapsari et al., 2024). Research conducted by Ansel et al. (2016) showed that tamanu oil emulsion accelerates wound closure in both keratinocyte and fibroblast cells, surpassing the effects of vitamin C (positive control). It not only stimulates cell proliferation but also increases glycosaminoglycan and collagen production, demonstrating its comprehensive wound-healing capabilities. They also performed a transcriptomic analysis, which further revealed that tamanu oil modulates genes associated with metabolic processes, O-glycan biosynthesis, cell adhesion, and proliferation, underscoring its potential to enhance the wound healing process significantly. Nguyen et al. (2017) revealed that topical application of calophyllolide, the main compound in *Calophyllum inophyllum*, significantly accelerates the skin wound healing process in rats. They conducted experiments by creating wounds on the backs of rats, administering three types of treatments (topical calophyllolide, povidone-iodine, and vehicle), and monitoring the wound progress for 14 days. The results showed that calophyllolide was more effective in speeding up wound healing compared to povidone-iodine and the vehicle (PBS-treated) on days 7 and 14 post-treatment. Histological analysis on day 14 also revealed reduced fibrosis and increased wound healing in the calophyllolide group. These findings indicate that calophyllolide has the potential to reduce fibrosis and enhance the wound-healing process through anti-inflammatory mechanisms. Erdogan et al. (2021) also highlight the substantial potential of tamanu oil in cutaneous wound healing. In their study, biopsies were obtained from rat wound sites, and the healing process was observed for 21 days to assess wound contraction. The findings indicated a significant reduction in wound contraction in the group treated with tamanu oil compared to both the control group and other treatment groups. By the seventh day, there was a noticeable increase in macrophage infiltration and the formation of mature granulation tissue in the tamanu oil and Centella groups compared to the control group. Furthermore, fibrosis and collagen density were notably higher in the tamanu oil group by the seventh day compared to the other groups, emphasizing its potential for promoting effective wound healing.

Tamanu oil contains a variety of compounds such as calophyllolide, calophyllic acid, inophyllum B, inophyllum C, and inophyllum E, along with polyphenols that possess antioxidant properties. The combination of these compounds plays a crucial role in the wound healing process, including reducing inflammation, enhancing healing processes, and supporting overall skin health. Additionally, tamanu oil is known for its potent anti-inflammatory activity, antioxidant effects that shield the skin from free radicals, and antimicrobial properties (Tran et al., 2024). Among the compounds contributing significantly to these activities are flavonoids like calophyllolide, inophyllide, and calophyllic acid. Calophyllolide, in particular, has been further studied to understand its role in controlling inflammatory reactions and tissue swelling at the cellular level, indicating its potentially positive effects in promoting wound healing. Dweck & Meadows (2002) reports that calophyllolide and inophyllide can reduce edema by 60,7% and 29,8%, respectively, compared to hydrocortisone (44%), suggesting that tamanu oil can be an effective natural solution in alleviating inflammation and accelerating the skin healing process. Furthermore, tamanu oil is rich in both saturated and unsaturated fatty acids. According to research conducted by Balitbang Kehutanan (2018), primary fatty acid components of tamanu oil include oleic acid (37.57%), linoleic acid (26.33%), stearic acid (19.96%), and palmitic acid (14.60%). It also contains smaller amounts of arachidic acid (0.94%), erucic acid (0.72%), linolenic acid (0.27%), and myristic acid (0.09%). These diverse fatty acids play crucial roles in the oil's pharmacological properties, contributing to its anti-inflammatory, antimicrobial, and wound-healing effects.

In this study, the visual assessment of tamanu oil produced through the cold-pressing method revealed a distinctively brighter hue compared to oil obtained via hot pressing. Subsequent GC analysis corroborated these observations by demonstrating elevated levels of various fatty acids in the cold-pressed tamanu oil, except palmitic acid, which showed a higher concentration in the hot-pressed oil. These findings aligned with prior research conducted by Balitbang Kehutanan (2018), where the predominant fatty acids were oleic acid, followed by linoleic acid, stearic acid, and palmitic acid. Further analysis was conducted using acid and peroxide values to assess the quality of fatty acids. The acid value measures the amount of free fatty acids present in the oil, with higher values indicating a significant content of free fatty acids that can reduce the quality of oil due to mucilage and sludge formation (Tavakoli et al., 2024). Meanwhile, the peroxide value indicates the level of oil damage due to oxidation. An increase in peroxide value can accelerate the formation of rancid odors in the oil. A value exceeding 100 meq peroxide/kg of oil indicates the toxicity and unpleasant odor of the oil. The increase in peroxide value serves as an indicator of the development of a disruptive rancid odor in the oil (Ghohestani et al., 2023). Cold-pressed tamanu oil has lower acid and peroxide values compared to the hot-pressed method. Research conducted by Mukhametov et al. (2023) demonstrated the impact of heat on the acid and peroxide values in vegetable oils. The gradual increase in temperature (ranging from 40°C to 75°C) correlated with elevated acid and peroxide values. This phenomenon can be attributed to the accelerated oxidation of triacylglycerols, leading to the formation of higher concentrations of unsaturated fatty acids, which subsequently contribute to the rise in acid values.

The results of the average wound length measurements were converted into percentages to depict the wound healing rate. The shorter the wound length, the higher the percentage of wound healing. On day 8, cold-pressed tamanu oil demonstrated the most effective wound healing rate among the various treatment groups. Each treatment group exhibited an increase in wound healing percentages from day 0 to day 8, indicating a progressive reduction in wound size each day. Wound recovery is a natural bodily process aimed at repairing damaged tissues, albeit with a potential for slow progression and susceptibility to infections (Abazari et al., 2022). This was evident in the notably lower percentage of wound recovery observed in the negative control group, which lacked compounds serving as wound coverings. In addition to the percentage of wound healing, the ability of tamanu oil as a wound cover was also evaluated based on the total AUC value. The AUC value correlates with the size of the incision wound. The smaller the wound, the lower the AUC value, indicating a more significant potential for the product as a wound healer. Cold-pressed tamanu oil showed the lowest AUC value, followed by the positive control and hot-pressed tamanu oil. The result was consistent with the fatty acid profile's result, where cold-pressed tamanu oil had lower acid and peroxide values, indicating better quality compared to hot-pressed tamanu oil. However, hot-pressed tamanu oil still showed significant differences compared to the negative control in terms of AUC value, confirming the ability of tamanu oil as a wound healing agent.

The study conducted by Pereira et al. (2008) revealed that oleic acid and linoleic acid can heal wounds by triggering a pro-inflammatory response. It was found that both fatty acids can increase wound mass, protein, and DNA contents (cellularity), as well as enhance neutrophil infiltration and cytokine production by neutrophils. From these findings, it is speculated that this mechanism is one of the reasons why oleic acid and linoleic acid can accelerate the wound healing process. Cardoso et al. (2011) investigated the immunomodulatory effect of oleic acid in wound recovery. The research observed increased collagen and pro-inflammatory cytokines in oleic acid-treated mice, alongside higher IL-10 levels and reduced COX2 gene expression. These results suggested that elevated IL-10 production may balance IL-17 effects, regulating inflammation and promoting quicker wound healing in oleic acid-treated wounds. These findings collectively explained reduced inflammation and faster wound closure in treated mice. Fatty acids could also influence tissue repair by affecting MMP balance, which is crucial for healing and tissue remodeling processes. The research conducted by Zhao et al. (2022) also revealed the ability of linoleic acid in wound healing. They examined the effect of linoleic acid on accelerating burn wound healing in pig skin. The results showed that wounds treated with linoleic acid healed faster than the negative control group, with a healing rate reaching 100% after 37 days, while the negative control group required 45 days. Further analysis using in vitro cell experiments also indicated that LA stimulated the proliferation of human keratinocytes and fibroblasts, which are the main stages of wound healing.

CONCLUSION

Cold-pressed tamanu oil exhibits superior fatty acid characteristics compared to hot-pressed tamanu oil. Moreover, within this investigation, cold-pressed tamanu oil demonstrated the highest level of wound healing efficacy compared to both the positive control and hot-pressed tamanu oil.

ACKNOWLEDGEMENT

This work was supported by the project RKAT PTNBH Universitas Sebelas Maret 2024 "PENELITIAN HIBAH GRUP RISET (PENELITIAN HGR-UNS) A, with a contract number of 194.2/UN27.22/PT.01.03/2024.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCE

Abazari, M., Ghaffari, A., Rashidzadeh, H., Badeleh, S. M., & Maleki, Y. (2022). A Systematic Review on Classification, Identification, and Healing Process of Burn Wound Healing. *The International Journal of Lower Extremity Wounds*, *21*(1), 18–30. https://doi.org/10.1177/1534734620924857

Agustin, R., Dewi, N., & Rahardja, S. . (2016). Efektivitas Ekstrak Ikan Haruan (*Channa Striata*) Dan Ibuprofen Terhadap Jumlah Sel Neutrofil Pada Proses Penyembuhan Luka Studi In Vivo Pada Mukosa Bukal Tikus (*Rattus Norvegicus*) Wistar. *DENTINO; Jurnal Kedokteran Gigi*, *1*(1), 68–74.

Ansel, J.-L., Lupo, E., Mijouin, L., Guillot, S., Butaud, J.-F., Ho, R., Lecellier, G., Raharivelomanana, P., & Pichon, C. (2016). Biological Activity of Polynesian *Calophyllum inophyllum* Oil Extract on Human Skin Cells. *Planta Medica*, *82*(11/12), 961–966. https://doi.org/10.1055/s-0042-108205

Balitbang Kehutanan. (2018). *Nyamplung (Calophyllum inophyllum L.) Sumber Energi Biofuel yang Potensial*. Badan Litbang Kehutanan Departemen Kehutanan.

Budiawan, A., Purwanto, A., Puradewa, L., Cahyani, E. D., & Purwaningsih, C. E. (2023). Wound Healing Activity and Flavonoid Contents of Purslane (*Portulaca grandiflora*) of various Varieties. *RSC Advances*, *13*(15), 9871–9877. https://doi.org/10.1039/D3RA00868A

Cardoso, C. R., Favoreto, S., Oliveira, L. L., Vancim, J. O., Barban, G. B., Ferraz, D. B., & Silva, J. S. (2011). Oleic Acid Modulation of the Immune Response in Wound Healing: A New Approach for Skin Repair. *Immunobiology*, *216*(3), 409–415. https://doi.org/10.1016/j.imbio.2010.06.007

Choudhary, V., Choudhary, M., & Bollag, W. B. (2024). Exploring Skin Wound Healing Models and the Impact of Natural Lipids on the Healing Process. *International Journal of Molecular Sciences*, *25*(7), 1–29. https://doi.org/10.3390/ijms25073790

Criollo-Mendoza, M. S., Contreras-Angulo, L. A., Leyva-López, N., Gutiérrez-Grijalva, E. P., Jiménez-Ortega, L. A., & Heredia, J. B. (2023). Wound Healing Properties of Natural Products: Mechanisms of Action. *Molecules*, *28*(2), 1–18. https://doi.org/10.3390/molecules28020598

Dweck, A. C., & Meadows, T. (2002). Tamanu (*Calophyllum inophyllum*) – the African, Asian, Polynesian and Pacific Panacea. *International Journal of Cosmetic Science*, *24*(6), 341–348. https://doi.org/10.1046/j.1467-2494.2002.00160.x

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*, 6–15. https://doi.org/10.12968/jowc.2021.30.Sup9a.V

Fan, P., Xie, S., Zhang, Z., Yuan, Q., He, J., Zhang, J., Liu, X., Liu, X., & Xu, L. (2024). *Polygonum perfoliatum* L. Ethanol Extract Ameliorates 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis-Like Skin Inflammation. *Journal of Ethnopharmacology*, *319*, 117288. https://doi.org/10.1016/j.jep.2023.117288

Feng, K., Tang, J., Qiu, R., Wang, B., Wang, J., & Hu, W. (2024). Fabrication of a core–Shell Nanofibrous Wound Dressing With An Antioxidant Effect On Skin Injury. *Journal of Materials Chemistry B*, *12*(9), 2384–2393. https://doi.org/10.1039/D3TB02911E

Ghanadian, M., Soltani, R., Homayouni, A., Khorvash, F., Jouabadi, S. M., & Abdollahzadeh, M. (2022). The Effect of

Plantago major Hydroalcoholic Extract on the Healing of Diabetic Foot and Pressure Ulcers: A Randomized Open-Label Controlled Clinical Trial. *The International Journal of Lower Extremity Wounds*, 1–7. https://doi.org/10.1177/15347346211070723

Ghohestani, E., Tashkhourian, J., & Hemmateenejad, B. (2023). Colorimetric determination of Peroxide Value in Vegetable Oils Using A Paper Based Analytical Device. *Food Chemistry*, *403*, 134345. https://doi.org/10.1016/j.foodchem.2022.134345

Gunawan, S., Pamungkas, B., Primaswari, C. S., Hapsari, S., & Aparamarta, H. W. (2020). Calophyllolide Separation from *Calophyllum inophyllum* Oil by Silica Gel Adsorption. *Materials Science Forum*, *988*(12), 101–107. https://doi.org/10.4028/www.scientific.net/MSF.988.101

Gunawan, Y., Pangkahila, A., & Darwinata, A. E. (2021). Topical Administration of Tamanu Oil (*Calophyllum inophyllum*) Inhibited the Increase of Matrix Metalloproteinase-1 (MMP-1) Expressions and Decrease of Collagen Dermis Amount in Male Wistar Rats Exposed to Ultraviolet B. *Neurologico Spinale Medico Chirurgico*, *4*(3), 114–118. https://doi.org/10.36444/nsmc.v4i3.186

Hapsari, S., Aparamarta, H.W., Jadid, N., & Gunawan, S. (2024). Extraction of Coumarin Mixture from Tamanu Oil using Food-Grade. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, *111*(2), 1–15. https://doi.org/10.37934/arfmts.111.2.115

Hasibuan, S., Sahirman, & Yudawati, N. M. A. (2013). Karakteristik Fisikokimia dan Antibakteri Hasil Purifikasi Minyak Biji Nyamplung (*Calophylum inophyllum* L.). *AGRITECH*, *33*(3), 311–319.

He, L., Di, D., Chu, X., Liu, X., Wang, Z., Lu, J., Wang, S., & Zhao, Q. (2023). Photothermal Antibacterial Materials to Promote Wound Healing. *Journal of Controlled Release*, *363*, 180–200. https://doi.org/10.1016/j.jconrel.2023.09.035

Krishnappa, M., Abraham, S., Furtado, S. C., Krishnamurthy, S., Rifaya, A., Asiri, Y. I., Chidambaram, K., & Pavadai, P. (2024). An Integrated Computational and Experimental Approach to Formulate Tamanu Oil Bigels as Anti-Scarring Agent. *Pharmaceuticals*, *17*(1), 1–15. https://doi.org/10.3390/ph17010102

Léguillier, T., Lecsö-Bornet, M., Lémus, C., Rousseau-Ralliard, D., Lebouvier, N., Hnawia, E., Nour, M., Aalbersberg, W., Ghazi, K., Raharivelomanana, P., & Rat, P. (2015). The Wound Healing and Antibacterial Activity of Five Ethnomedical *Calophyllum inophyllum* Oils: An Alternative Therapeutic Strategy to Treat Infected Wounds. *PLOS ONE*, *10*(9), 1–20. https://doi.org/10.1371/journal.pone.0138602

Liu, W.-H., Liu, Y.-W., Chen, Z.-F., Chiou, W.-F., Tsai, Y.-C., & Chen, C.-C. (2015). Calophyllolide Content in *Calophyllum inophyllum* at Different Stages of Maturity and Its Osteogenic Activity. *Molecules*, *20*(7), 12314–12327. https://doi.org/10.3390/molecules200712314

Maleki, H., Doostan, M., Khoshnevisan, K., Baharifar, H., Maleki, S. A., & Fatahi, M. A. (2024). *Zingiber officinale* and *Thymus vulgaris* Extracts Co-Loaded Polyvinyl Alcohol and Chitosan Electrospun Nanofibers for Tackling Infection and Wound Healing Promotion. *Heliyon*, *10*(1), 1–15. https://doi.org/10.1016/j.heliyon.2023.e23719

Morikawa, T., Nagatomo, A., Kitazawa, K., Muraoka, O., Kikuchi, T., Yamada, T., Tanaka, R., & Ninomiya, K. (2018). Collagen Synthesis-Promoting Effects of Andiroba Oil and its Limonoid Constituents in Normal Human Dermal Fibroblasts. *Journal of Oleo Science*, *67*(10), 1271–1277. https://doi.org/10.5650/jos.ess18143

Mukhametov, A., Dautkanova, D., Kazhymurat, A., Yerbulekova, M., & Aitkhozhayeva, G. (2023). The Effects of Heat Treatment on the Oxidation Resistance and Fatty Acid Composition of the Vegetable Oil Blend. *Journal of Oleo Science*, *72*(6), 597–604. https://doi.org/10.5650/jos.ess23010

Nachippan, N. M., Parthasarathy, M., Elumalai, P. V., Backiyaraj, A., Balasubramanian, D., & Hoang, A. T. (2022). Experimental Assessment on Characteristics of Premixed Charge Compression Ignition Engine Fueled with Multi-Walled Carbon Nanotube-Included Tamanu Methyl Ester. *Fuel*, *323*, 124415. https://doi.org/10.1016/j.fuel.2022.124415

Nguyen, M. N., Le, T. D., Nguyen, B. V., Nguyen, T. N.

L., Pioch, D., & Mai, H. C. (2021). Purification Trials of Tamanu (*Calophyllum inophyllum* L.) oil. *OCL*, *28*, 53–60. https://doi.org/10.1051/ocl/2021042

Nguyen, V.-L., Truong, C.-T., Nguyen, B. C. Q., Vo, T.-N. Van, Dao, T.-T., Nguyen, V.-D., Trinh, D.-T. T., Huynh, H. K., & Bui, C.-B. (2017). Anti-Inflammatory and Wound Healing Activities of Calophyllolide Isolated from *Calophyllum inophyllum* Linn. *PLOS ONE*, *12*(10), 1–16. https://doi.org/10.1371/journal.pone.0185674

Pathak, P. C., & Gadgoli, C. H. (2024). Exploring the Efficacy of Panchavalkal Extract And Zinc-Copper Bhasma in Promoting Wound Healing in Incision and Excision Wound Models in the Rat. *Journal of Ethnopharmacology*, *320*, 117404. https://doi.org/10.1016/j.jep.2023.117404

Pelin, I. M., Silion, M., Popescu, I., Rîmbu, C. M., Fundueanu, G., & Constantin, M. (2023). Pullulan/Poly(vinyl alcohol) Hydrogels Loaded with Calendula officinalis Extract: Design and In Vitro Evaluation for Wound Healing Applications. *Pharmaceutics*, *15*(6), 1–27. https://doi.org/10.3390/pharmaceutics15061674

Pereira, L. M., Hatanaka, E., Martins, E. F., Oliveira, F., Liberti, E. A., Farsky, S. H., Curi, R., & Pithon-Curi, T. C. (2008). Effect of oleic and Linoleic Acids on the Inflammatory Phase of Wound Healing in Rats. *Cell Biochemistry and Function*, *26*(2), 197–204. https://doi.org/10.1002/cbf.1432

Qiu, X., Nie, L., Liu, P., Xiong, X., Chen, F., Liu, X., Bu, P., Zhou, B., Tan, M., Zhan, F., Xiao, X., Feng, Q., & Cai, K. (2024). From Hemostasis to Proliferation: Accelerating the Infected Wound Healing Through a Comprehensive Repair Strategy Based on GA/OKGM Hydrogel Loaded with MXene@TiO2 Nanosheets. *Biomaterials*, *308*, 122548. https://doi.org/10.1016/j.biomaterials.2024.122548

Saeidi, S., Ghanadian, S. M., Poostiyan, N., & Soltani, R. (2024). Evaluation of the effectiveness of *Berberis integerrima* Bunge Root Extract Combined with Spearmint Essential Oil In The Treatment of *Acne vulgaris*: A Randomized Controlled Clinical Trial. *Journal of Cosmetic Dermatology*. https://doi.org/10.1111/jocd.16291

Sanpinit, S., Chokpaisarn, J., Na-Phatthalung, P., Sotthibandhu, D. S., Yincharoen, K., Wetchakul, P., Limsuwan, S., & Chusri, S. (2024). Effectiveness of Ya-Samarn-Phlae in Diabetic Wound Healing: Evidence from In Vitro Studies and a Multicenter Randomized Controlled Clinical Trial. *Journal of Ethnopharmacology*, *326*, 117929. https://doi.org/10.1016/j.jep.2024.117929

Tavakoli, J., Ghorbani, A., Hematian Sourki, A., Ghani, A., Zarei Jelyani, A., Kowalczewski, P. Ł., Aliyeva, A., & Mousavi Khaneghah, A. (2024). Thermal Processing of Pomegranate Seed Oils Underscores Their Antioxidant Stability and Nutritional Value: Comparison of Pomegranate Seed Oil With Sesame Seed Oil. *Food Science & Nutrition*, *12*(3), 2166–2181. https://doi.org/10.1002/fsn3.3918

Tran, T. P. A., Luong, A. H., & Lin, W.-C. (2024). Characterizations of Centrifugal Electrospun Polyvinyl Alcohol/Sodium Alginate/Tamanu Oil/Silver Nanoparticles Wound Dressing. *IEEE Transactions on NanoBioscience*, *23*(2), 368–377. https://doi.org/10.1109/TNB.2024.3371224

Yimdjo, M. C., Azebaze, A. G., Nkengfack, A. E., Meyer, A. M., Bodo, B., & Fomum, Z. T. (2004). Antimicrobial and Cytotoxic Agents from *Calophyllum inophyllum*. *Phytochemistry*, *65*(20), 2789–2795. https://doi.org/10.1016/j.phytochem.2004.08.024

Zhao, D., Xiao, J., Qiang, L., Deng, X., An, J., Zhang, Q., Zhao, F., Ma, J., Fang, C., Guan, G., Wu, Y., & Xie, Y. (2022). Walnut Ointment Promotes Full-Thickness Burning Wound Healing: Role of Linoleic Acid. *Acta Cirúrgica Brasileira*, *37*(9). https://doi.org/10.1590/acb370902

Zhao, Y., Zhu, L., Yang, L., Chen, M., Sun, P., Ma, Y., Zhang, D., Zhao, Y., & Jia, H. (2024). In vitro and in vivo anti-eczema effect of *Artemisia annua* Aqueous Extract and Its Component Profiling. *Journal of Ethnopharmacology*, *318*, 1–14. https://doi.org/10.1016/j.jep.2023.117065