

## **Tamanu oil (*Calophyllum inophyllum* L) promotes wound-healing activity in alloxan-induced diabetic rats, and its fatty acids profile**

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### **Abstract**

Diabetes Mellitus (DM) is a metabolic disease and is a severe threat because it causes diabetic wounds. Treating injuries in patients with DM is dominated by synthetic chemicals such as anti-inflammatory, corticosteroids, and antibacterial. It has side effects, so further exploration of natural ingredients is needed. One plant that has the potential to heal wounds is tamanu oil or nyamplung oil. This study aimed to investigate the effect of tamanu oil on the healing of diabetic wounds and the fatty acid composition profile. Male rats of the Wistar strain (*Ratus norvegicus*) were given incision wounds along a 2 cm depth of 2 mm, randomly divided into three groups, by applying topically twice a day. We are profiling fatty acids using gas chromatography-mass spectrometry (GC-MS). As far as we know, this is the first study showing that tamanu oil can speed up the healing of diabetic wounds in rats. The Tamanu oil containing oleic acid (42.65%), linoleic acid (25.69%), stearic acid (16.50%), and palmitic acid (12.93) had better effectiveness than other groups, demonstrated by wound closure exceeding 50% on day six and wound closure by 100% on day nine.

**Keywords:** Bioactive; Diabetic Rats; Fatty Acids; Nyamplung; Wound Healing

### **1. INTRODUCTION**

Diabetes Mellitus (DM) is included in the top 10 causes of death globally. The International Diabetes Federation (IDF) reported that as of May 2020, there are 463 million people in the world suffering from DM, with a global prevalence reaching 6.12% (International Diabetes Federation, 2021). DM threatens health development in Indonesia because it can cause blindness, kidney failure, heart disease, stroke, infection, slowed wound healing, and diabetic foot ulcers (gangrene), so they must be amputated and cause chronic wound growth (Kementerian Kesehatan Republik Indonesia, 2013; Uckay et al., 2014). Synthetic chemicals such as anti-inflammatories, corticosteroids, and antibiotics dominate wound treatment in patients with DM. Synthetic drugs have many shortcomings, such as relatively high prices, side effects, and somewhat less effectiveness in treating chronic wounds of DM sufferers (Pereira and Paulo, 2016). Therefore, another way is needed to treat wounds in diabetic patients optimally.

One plant that has the potential to heal wounds is tamanu oil or nyamplung oil (Java). Tamanu is obtained from cold-pressed and used as a carrier oil (Ansel et al., 2016). The benefits of tamanu oil include anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, photoprotective, and wound healing (Bhalla et al., 1980; Yimdjo et al., 2004; Saravanan et al., 2011). Nguyen and Trans (2016) reported nine saturated fatty acids (caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric) and 9 unsaturated fatty acids (oleic acid, linoleic acid,  $\alpha$ -linolenic,  $\gamma$ -linolenic, eicosenoic, arachidonic, eicosapentaenoic, erucic, docosahexaenoic) which were identified in tamanu oil and these compounds inhibit some strains *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, MRSA ATCC 43300, *P. aeruginosa* ATCC 277853 and anti-inflammatory agent. Besides fatty acids, polyphenols, calophyllolide, inophyllum, and calophyllic acids are the chemical constituents of *C. inophyllum* L that are also involved in wound healing functions (Dweck et al., 2002). Based on research by Erdogan et al. (2021), which assessed the ability of tamanu oil to cure skin wounds, demonstrated that the tamanu and Centella groups had considerably higher levels of mature granulation tissue and macrophage infiltration than the control group.

Furthermore, the tamanu group had higher concentrations of collagen and fibrosis than the other groups. Research by Krishnappa et al. (2024) also revealed that tamanu oil markedly enhances wound healing and minimizes scarring in Wistar rats within 15 days. The group treated with tamanu oil exhibited complete wound closure with minimal scar formation, in contrast to other treatment groups that required a longer duration for healing. Although various studies have documented the wound-healing properties of tamanu oil, none have specifically explored its potential in treating diabetic wounds despite its known antibacterial and anti-inflammatory properties. Therefore, this study aims to examine the effects of tamanu oil on diabetic wound healing and to analyze its fatty acid profile through gas chromatography.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The study subjects were 18 white rats of the male Wistar strain with an average weight of 200g, aged 1.5-2 months. Approval was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital, Number 1.690/IX/HREC/2023. Tamanu oil (CV. Plantanesia), commercial product<sup>®</sup> (Kimia Farma) gel, alloxan monohydrate, *scalpel* (B-Braun), and *alcohol swab*.

### 2.2 Methods

The study was conducted experimentally at the Integrated UPT Biology Sub-Laboratory, Sebelas Maret University, Surakarta. The subjects were divided randomly into three groups: the negative control group (no treatment), the positive control (commercial), and the tamanu oil group, applying twice daily. Test animals were acclimatized before treatment. Rats induced diabetes subcutaneously with Alloxan monohydrate at a dose of 120 mg/kg body weight and tested blood sugar levels seven days after induction. Glucose levels in rats were measured using

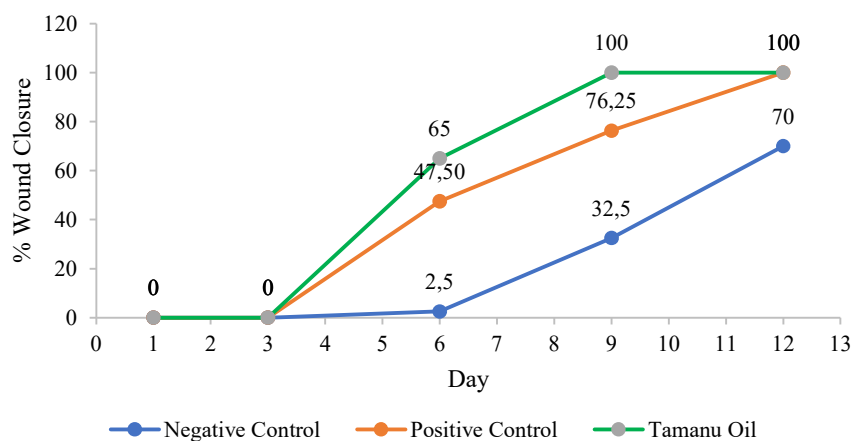
a glucometer before and after alloxan monohydrate induction. Diabetes was identified in rats when their blood glucose levels were greater than 126 mg/dL. (Rahmawati et al., 2014). Incised wounds were made 2 cm long with a depth of 2 mm on the back of the rat's back using a scalpel. The parameter observed was the average percentage of wound length during the 12 days of observation. The fatty acid content of tamanu oil was analyzed using a high-performance gas chromatography system (Hawlet, USA) with an 18-6-1/MU/SMM-SIG method. The fatty acid profile was determined through gas chromatography (GC) utilizing a DB FastFAME column and a flame ionization detector (FID). Helium served as the carrier gas. The sample, injected in split mode at 240 °C with a volume of 1  $\mu$ L, was analyzed with the oven temperature initially set at 50 °C and then gradually raised to 230 °C (Romulo and Sadek, 2022).

### 3. RESULTS AND DISCUSSION

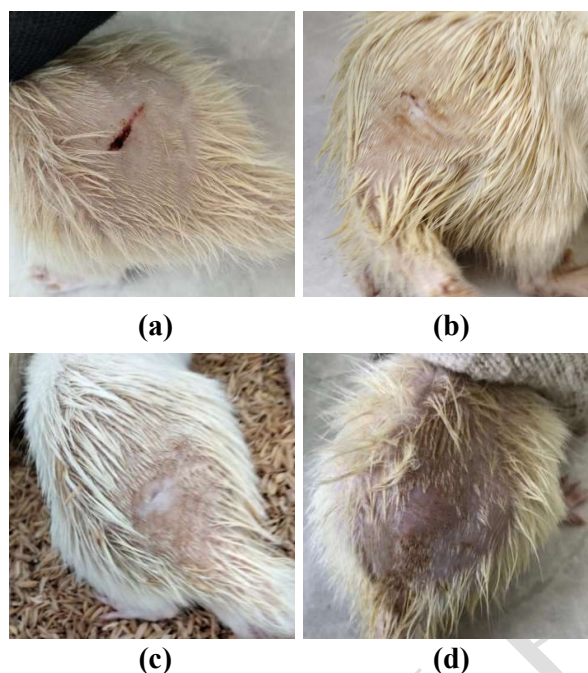
#### 3.1. Wound healing activity

The average blood sugar of rats induced by alloxan monohydrate is 158.83mg/dL, which shows that the induction of alloxan monohydrate has been successful. The rats are characterized by the clinical condition of rats in the form of lethargy and inactivity as before compared to controls. Compared to the other groups during a 12-day observation period, the test findings demonstrated that tamanu oil had activity as diabetic wound healing in terms of the percentage of wound healing (Figure 1).

Based on observations conducted on days 3, 6, and 9, the statistical results indicated no significant difference ( $p$ -value  $<0.05$ ) in the percentage of wound healing between the tamanu oil group and the other groups. Statistical testing was then used for Kruskal Wallis due to sig.  $<0.05$ . Tamanu oil resulted in a wound healing percentage of up to 50% on day six and wound closure of 100% on day 9. It showed that the effectiveness of wound healing was better than the other control groups. Figure 2 depicts the appearance of the wound size in rats given tamanu oil application for a period of 12 days.



**Figure 1.** The percentage of wound closure for 12 days of the negative control group (no treatment), the positive control (commercial drug), and the tamanu oil.



**Figure 2.** Observation of Wound Length of Rats Giving Tamanu Oil. Description: Day 3 (a); Day 6 (b); Day 9 (c); Day 12 (d).

### 3.2. Fatty acid profile of tamanu oil

Some fatty acids were identified in tamanu oil (Table 1). The fatty acid profile of the Tamanu oil in our investigation and relevant findings from the literature are shown in Table 1. The results showed that the primary fatty acids identified in Tamanu oil were oleic acid (42.65%), linoleic acid (25.69%), stearic acid (16.50%), and palmitic acid (12.93%). We observed that the high content of oleic acid and linoleic acid was in close agreement with the results from other researchers (Crane et al., 2005; Do, 2022; Nguyen and Tran (2016)). The remarkably high content of those components makes tamanu oil a potential plant source of Omega-6 and Omega-9. Omega, a blend of vital fatty acids, is among tamanu oil's most significant constituents, aiding in healing several skin wounds and cell regeneration. Linoleic acid, an omega-6 fatty acid, has anti-inflammatory and analgesic properties, healing injuries and treating skin diseases (Nguyen and Tran, 2016). Fatty acids may play a role in promoting the healing of skin wounds, according to several studies (Léguillier et al., 2015; Jara et al., 2020). Oleic acid also could increase the synthesis of CINC-2a/b, a cytokine-induced neutrophil chemoattractant. Therefore, oleic and linoleic acids have a pro-inflammatory action that may hasten the healing of wounds (Pereira et al., 2008). Rodrigues et al. (2016) revealed that linoleic acid decreased the area of the wound in diabetic rats after 14 days of wound induction. Moreover, it decreased the production of macrophage inflammatory protein-1 (MIP-1) and macrophage chemoattractant protein-1 (MCP-1) and elevated the amounts of leukotriene B4 (LTB4), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and cytokine-induced neutrophil chemotaxis (CINC-2 $\alpha\beta$ ).

In this study, the test results show that tamanu oil can be used to treat diabetic wounds. The wound-healing ability of tamanu oil emulgel is caused by the presence of bioactive compounds that play a role in the wound-healing process, including calophyllolide, innohyllide, oleic acid, linoleic acid, saponins, triterpenes (Dweck and Meadows, 2002; Min Oo, 2018; Nguyen and Tran, 2016). Oleic and linoleic acid-containing fatty acid mixes have been utilized in Brazil to prevent and cure pressure ulcers (Jara et al., 2020). The process of wound healing is dynamic and consists of four phases: wound remodeling with the creation of scar tissue, inflammation, coagulation and hemostasis, and proliferation. However, bacterial contamination poses a serious threat to the healing process of wounds. A bacterial infection can result in a chronic inflammatory state for the wound. Thus, if adequate treatment is not given, the wound may become nonhealing or chronic (Nguyen et al., 2017). According to research by Nguyen et al. (2017), *Calophyllum inophyllum* oil (CIO) has wound healing and antibacterial effects in vitro tests on HaCaT cells. The concentration of CIO needed to prevent wound healing and bacterial growth is less than the concentration of CIO, which causes keratinocyte cells to become cytotoxic, supporting the topical use of CIO. Other studies have shown that Tamanu Oil Emulsion (TOE) can increase collagen production when tested for its healing effect on keratinocyte (HaCaT) and fibroblast (HDF) cells and can accelerate the wound closure process in the scratched fibroblast monolayer layer (Ansel et al., 2016). The methanol extract of *C. inophyllum* showed potent antibacterial activity ( $17 \pm 1.73$  to  $24 \pm 1.15$  mm) against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. It was identified as 4Pentan-2-ol -(3-methylazetid-1-yl) (Anjukam et al., 2023). Tamanu oil also showed an antibacterial effect on *Propionibacterium acnes* ( $19,67 \pm 0,47$  mm) (Artanti et al., 2020). In addition to the tamanu oil, it has been documented that every component (leaves, bark, fruits, and fruit peels) possesses antibacterial qualities against a range of gram-positive and gram-negative bacteria, including *Bacillus coagulans*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. (Ha et al., 2009; Mishra et al., 2010; Kumar and Garg, 2020; Liang et al., 2022). Consistent with previous findings, the primary component of *Calophyllum inophyllum*, calophyllolide, enhanced the healing of cutaneous wounds (Nguyen et al., 2017). Based on this potential, tamanu oil can be developed to treat diabetic wounds. We suggested that other dosage forms can be developed further for topical use.

**Table 1.** The fatty acid component in tamanu oil.

Components		This study content (%)	Do's study (Do, 2022)	Sylvie Crane's study (%) (Crane et al., 2005)	Nguyen's study (Nguyen & Tran, 2016)
Oleic acid	C18:1	42.65	41.88	39.10	37.66
Linoleic acid	C18:2	25.69	29.94	31.10	33.86
Stearic acid	C18:0	16.50	13.52	14.30	13.25
Palmitic acid	C16:0	12.93	12.69	13.70	13.09

#### 4. CONCLUSION

Tamanu oil containing oleic acid, linoleic acid, stearic acid, and palmitic acid, when applied topically, helps accelerate the healing of diabetic wounds. Tamanu oil could heal wounds faster and more effectively than the other groups. This work presents the potential of tamanu oil to promote diabetic wound healing in rats, which could lead to the development of new applications for the oil in medicine.

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#### CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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