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Original Research Article

The Therapeutic Potential of Aceh Patchouli Oil (*Pogostemon cablin* Benth.) in Enhancing Full-Thickness Wound Healing in Mice

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ABSTRACT

Patchouli (*Pogostemon cablin*) oil is widely recognized for antifungal, anti-inflammatory, and antibacterial properties. This oil also has the potential to expedite collagen production and facilitate epithelialization, which are essential for wound healing. Furthermore, epithelialization comprises the formation of epithelial cells at wound edges, typically in 3 to 14 days after the injury. Several reports have shown that this process is facilitated by the presence of bioactive components, such as patchouli alcohol, β-patchoulene, β-farnesene, α-guanine, flavonoids, and saponins from patchouli oil. Therefore, this study aims to determine the effect of Aceh patchouli oil on epithelialization in full-thickness wound of *Mus musculus*. A total of 28 male mice received incision wound and were divided into four groups, which were given crude patchouli oil (CPO) (Group 1), light fraction of patchouli (CPO1) (Group 2), heavy fraction of patchouli (CPO2) (Group 3), and lanolin (Control/Group 4). Transparent dressings were then used for the daily wound care regimen, followed by comprehensive histopathological examinations to assess the outcomes after 15 days. The results showed that the average thickness of the skin epithelium formed with the application of CPO, CPO1, CPO2, and control was 128.4 μ, 127.7 μ, 84.9 μ, and 126.0 μ, respectively. ANOVA analysis showed a remarkable increase in epithelial thickness in groups 1 and 2 compared to 3. This showed that the light fraction of patchouli oil had significant potential for enhancing wound healing compared to heavy fraction.

Keywords: Angiogenesis, Collagen, Epithelization, Patchouli oil, *Pogostemon cablin* Benth, Wound healing.

Introduction

Wound is an injury that compromises the structural integrity of biological tissues, comprising the skin, mucous membranes, and organ tissues.[1] Wounds can result from various causes such as cuts, burns, or abrasions, and prompt and proper wound care is essential for effective healing and prevention of infections. Furthermore, it can be broadly categorized as either acute or chronic based on the healing duration, and further classified as partial or full thickness depending on the layers affected.[2,3] The process of wound healing is a natural and precisely orchestrated biological phenomenon in the human body, which typically occurs through four distinct phases. These include inflammatory (0-3rd day), proliferation (3rd-14th day), and tissue remodeling (from the 8th day, extending up to 1st year) phases.[1,3] According to previous studies, wound healing is a complex activity that comprises continuous interactions among cells, as well as between cells and the extracellular matrix.

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Consequently, the development of wound healing agents has predominantly focused on modulating cell and cytokine responses. The use of natural agents has gained prominence in several countries, particularly in Asia, Europe, and America, due to the biocompatibility and reduced toxicity. In Indonesia, several natural ingredients have been employed in wound care.[4,5] Herbal plants, specifically those identified to have compounds beneficial in preventing or treating diseases and performing specific biological functions.[6,7] A typical example is Patchouli, an annual herbaceous plant reaching a height of 0.5-1 m, which possesses fibrous roots, smooth serrated oval-shaped leaves, and a slightly pale color. The underside of the leaves and twigs contains fine hairs, and it has a woody trunk with a diameter of 10-20 mm. Several studies reported that the plant was recognized for producing essential oil known as patchouli oil.[8,9]

Patchouli is an herbal plant that produces essential oil with reported antibacterial, antifungal, and anti-inflammatory properties, contributing to accelerated collagen formation during wound healing.[10,11] The primary components of patchouli oil include eugenol, cinnamaldehyde, benzaldehyde, and saponins, which have been reported to play an essential role in the bioactivities.[12] Furthermore, Aceh patchouli plant exhibits a high concentration of patchoulol, flavonoids, and antioxidants, making it a promising alternative for wound care with the right formulation. These compounds inhibit the growth of bacteria, such as Bacillus subtilis and Staphylococcus aureus, commonly found in open wounds. According to previous studies, it contains saponin, which serves as a cleanser and expedites collagen formation.[13,14] Collagen, a macromolecular protein, comprises 20-30% of the total protein content

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in organisms. This protein also serves as the fundamental structural component of the extracellular matrix in all connective tissues, playing a crucial role, particularly in human skin.[15,16,17]

Epithelialization is the formation of epithelium, which comprises cell proliferation and migration at the wound edges in 3 to 14 days after injury.[18] This process has been reported to play an essential role in healing and serves as a key criterion for determining the successful closure of wound. In the absence of re-epithelialization, wound cannot be considered fully healed.[19] Various bioactive components derived from the patchouli oil have been hypothesized to play a role in stimulating this process. Therefore, this study aims to determine the effect of crude, light fraction, and heavy fraction patchouli oil on epithelialization in full-thickness wounds in male mice.

Materials and Methods

Plant collection and identification

Patchouli leaves (Figure 1) were collected from plants aged between 5 months to 2 years in February 2023. The identification process of P. cablin comprised observing the distinctive features, including appearance, scent, and growing conditions. The collected patchouli leaves were identified by Nadia Isnaini from Department of Pharmacy, Universitas Syiah Kuala. Furthermore, the voucher specimen was deposited at the Atsiri Research Center, Universitas Syiah Kuala (voucher number ARC-012023).

Plant extraction

The harvested leaves, as much as 5.6 kg, were dried and distilled by using steam distillation method to extract crude patchouli oil (CPO). The CPO was then fractionated into light and heavy fractions through a process known as fractional distillation through a vacuum rotary evaporator. The detailed procedure was described in research conducted by Desiyana et al.[11] This process involves heating the crude oil to a controlled temperature, causing its components to vaporize at different temperatures. The vapors rise through the fractionating column, which contains several trays or plates with varying temperatures. This fractionation led to the production of light fraction (CPO1) and heavy fraction (CPO2) of patchouli oil. The temperature settings ranged between 115°C and 160°C for heavy fraction, and 125°C and 160°C for light fraction. The fractionation process in this study was conducted under a pressure condition of 200 kPa.

Gas chromatography-mass spectrometry analysis

Essential oil was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis at the Instrumentation Laboratory of the Chemistry Department, FMIPA, USK, using a Shimadzu GC-2010 plus gas chromatography. Furthermore, the analysis was carried out for 50 minutes and employed a TG-5MS capillary column (30 meters in length, 0.2 mm inner diameter, with 0.25 μm film thickness). A 2 μL volume of essential oil was then introduced into GC instrument, which was initially set at 60°C for 4 minutes. Subsequently, the temperature was raised to 150°C for an additional 4 minutes and further increased to 250°C. The flow rate of the helium carrier gas was maintained at 1.35 mL/min. For MS measurements, ionization was carried out using electron impact at 70 eV. The resulting peaks were then cross-referenced with a mass spectrum database using specialized Chromeleon software designed for the interpretation of mass spectral fragmentation patterns.

In vivo full-thickness wound healing design

This study used a randomized post-test-only control group design, which administered several types of *P. cablin* oil to heal full-thickness wounds in mice with ethical clearance for animals. A total of 28 male mice aged 12 weeks with a body weight range of 25-30 grams, were divided into 4 treatment groups, namely CPO (Group 1), CPO1 (Group 2), CPO2 (Group 3), and lanolin as control (Group 4). The wound was made on the back area of mice body, followed by intramuscular anesthetization using ketamine 20 mg/kg body weight. Furthermore, the fur on the back of each mouse was shaved. Disinfection was carried out with 10% povidone-iodine solution and Savlon (1:30). A full-thickness wound measuring 1 x 1 cm was made using a scalpel, treated once every day using cotton buds according to the type of treatment for each group, and covered with a transparent dressing. Wound care was performed daily and a histopathological examination was carried out on the 15th day.

Statistical analysis

The data obtained in this study were presented in the form of tables, comprising sample numbers, treatments, and observation results containing data on epithelial thickness, collagen production, and angiogenesis on the 7th day. Furthermore, analysis was carried out using the Kruskal-Wallis test, followed by Mann-Whitney. The data were presented as mean ± SD and all analyses were performed using SPSS software for Windows version 26.0 (Chicago), with a significance level set at $p < 0.05$.

Figure 1: The leaves of *Pogostemon cablin* Benth.

Results and Discussion

Identified compounds from patchouli oil

Steam distillation was used to extract crude oil from patchouli plant, followed by purification with a rotary vacuum evaporator to obtain both light and heavy fractions of patchouli oil. Light fraction comprised 20-30% patchouli alcohol, while heavy fraction contained 60-70%. The results showed that administering patchouli oil at a concentration of 10% had the potential to be highly effective in inhibiting inflammation compared to other concentrations.[20] Furthermore, this study used graded doses to obtain the right concentration that could provide optimal anti-inflammatory effects.

Figure 2 shows the GC-MS chromatogram of PCO, PCO1, and PCO2. Anti-inflammatory activity of the sample could be attributed to the presence of isolated patchouli alcohol and β-patchoulene.[20] GC-MS results showed that light fraction had a higher β-patchoulene content and a lower patchouli alcohol compared to heavy fraction.[20,21] Furthermore, the alpha guanine content, which played a role in antibacterial activity in light fraction was found to be higher compared to heavy fraction. This explained the thickness of epithelialization in the groups given light fraction of patchouli. Chemical compositions of PCO, PCO1, and PCO2 are given in Table 1, Table 2, and Table 3, respectively. Light fraction influenced wound healing by increasing epithelialization more effectively compared to heavy fraction. However, no study reported the association between the effect of β-patchoulene and alpha guanine in increasing epithelialization of full-thickness wound healing.

In vivo full-thickness wound healing

Epithelialization was the formation of epithelium, comprising cell proliferation and migration at wound edges in 3 to 14 days after injury. This process was a critical factor in wound healing and served as a key indicator of successful wound closure.[22] In this study, there was an increase in epithelial thickness in the group given CPO and CPO1 compared to the control, but this increased value was not statistically significant. This was consistent with the theory, stating that patchouli oil had anti-microbial effect and could enhance epithelialization process of wound healing.[23,24]

Bioactive components contained in patchouli essential oil included patchouli alcohol, which had been reported to have antibacterial properties.[25] A previous showed that patchouli oil contained 3 compounds with a slightly higher antibacterial effect compared to benzylpenicillin, including patchouli alcohol, alpha-guaiene, and β-farnesene.[26] Furthermore, these 3 components caused damage to bacterial cell walls with similar effectiveness as benzylpenicillin. Administering light fraction patchouli oil could facilitate the wound healing process through antioxidant, anti-inflammatory, and anti-proliferative effects, thereby mitigating oxidative damage. Anti-inflammatory activity could possibly be due to the presence of patchouli alcohol and β-patchoulene isolated from patchouli oil.[27,28] The phenol functional group showed that CPO1 also had a high concentration of flavonoids and could be used as an antioxidant. The alcohol functional group (–OH) appeared at a wavelength of 3503-3313 cm-1, showing the presence of patchoulol.[29] Several studies showed that patchoulol was responsible for patchouli oil distinctive aroma and comprised oxygenated sesquiterpenes, which could also be used as antioxidant.[25,26]

The results of epithelialization on day 15 microscopically using Hematoxylin Eosin (H&E) staining are presented in Figure 3. By utilizing an H&E solution, the thickness of epithelialization was demarcated in blue, as shown by the red arrow.

Table 4 presents the thickness of epithelialization (in microns) for each treatment group. The results showed an increase in epithelialization thickness in the groups treated with CPO and CPO1, while a decrease was observed in samples treated with CPO2. To obtain a more comprehensive understanding of these variations, a descriptive statistical test was carried out to assess the average epithelial thickness. This was achieved by using 7 male mice for each group and then comparing the obtained results. The analysis result showed differences in the average thickness of epithelialization in each group.

Table 2: The chemical composition of light fraction patchouli oil (PCO1)

Retention time (min)	Identified compound	Relative area (%)
10.656	*β*-patchoulene	4.80
11.405	Trans caryophyllene	5.57
11.704	*α*-guaiene	13.43
12.170	Seychellene	8.89
12.427	*α*-patchoulene	6.59
12.486	*γ*-gurjune	2.48
13.058	*α*-guaiene	4.28
13.246	*δ*-guaiene	17.84
17.167	Patchouli alcohol	22.73

Table 3: The chemical composition of heavy fraction patchouli oil (PCO2)

Retention time (min)	Identified compound	Relative area (%)
12.086	*α*-patchoulene	1.46
12.735	*α*-guaiene	2.31
12.869	*δ*-guaiene	18.53
15.651	*α*-guaiene	0.73
16.333	*δ*-guaiene	3.43
16.575	*γ*-gurjunene	1.45
16.754	Patchouli alcohol	60.71
26.96	Silane	0.37

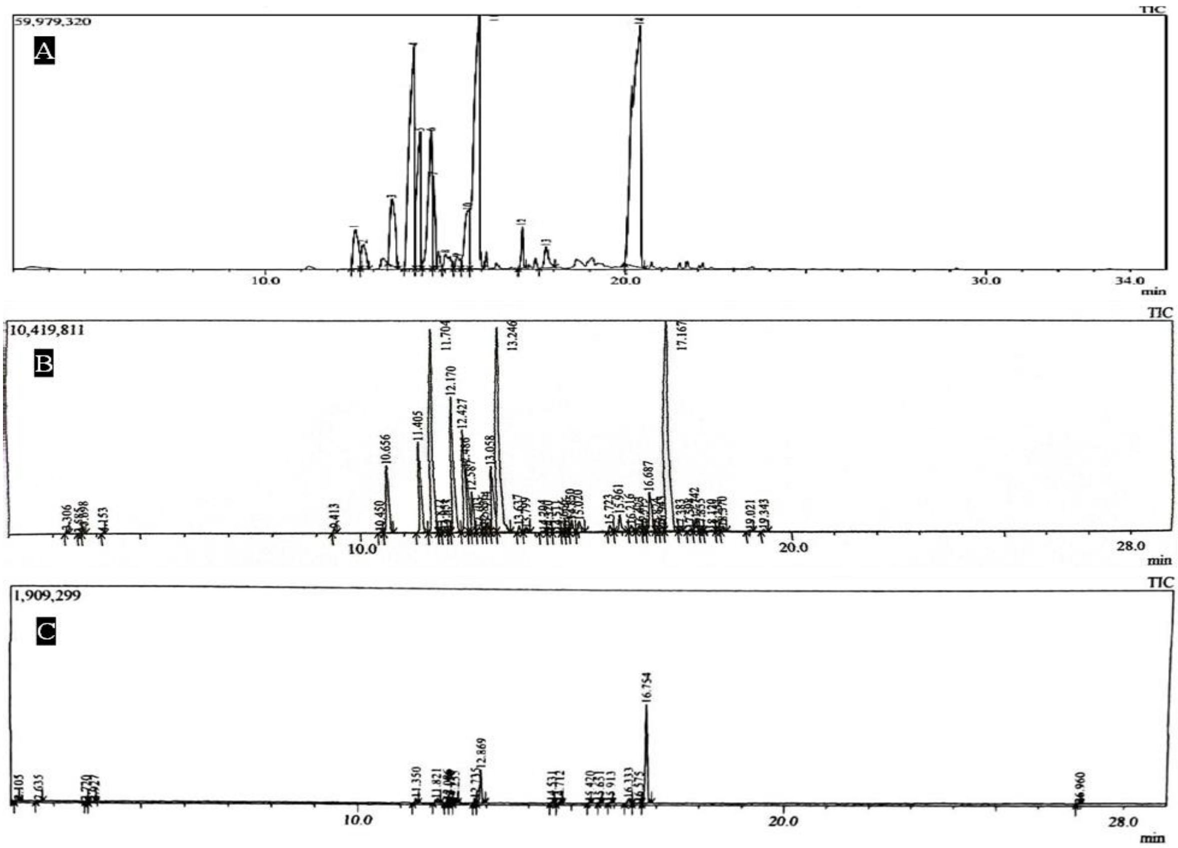

Figure 2: The GC-MS chromatogram of (A) PCO, (B) PCO1, and (C) PCO2

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Groups treated with crude and light fraction of patchouli oil exhibited high average epithelial thickness, while the lowest was observed in group treated with heavy fraction. The average thickness in the groups administered with crude and light fraction of patchouli oil surpassed that of the control group, while the samples treated with heavy fraction displayed a lower thickness, as shown in Figure 4. The resulting average value, measured in microns, for each group, was 128.4, 127.7, 84.9, and 126.0 for groups 1, 2, 3, and 4, respectively. This observed increase suggested the potential efficacy of raw patchouli oil and light fraction in accelerating wound healing process compared to heavy fraction.
Active components, such as saponins, tannins, flavonoids, and patchouli oil facilitated wound healing process, particularly in tissue formation.[30,31,32] This result was consistent with Gilaberte et al, which showed the positive impact of flavonoid content on wound healing, emphasizing the role in accelerating wound contraction, collagen deposition, and granulation tissue formation.[33] Saponin compounds were also found to facilitate the formation of new epithelial cells, thereby supporting the re-epithelialization process.[34] Although both CPO and light fraction had a comparable ability to enhance epithelial thickness in mice, the recommended choice for wound healing was light fraction. This was attributed to the lower impurities and iron content compared to CPO.

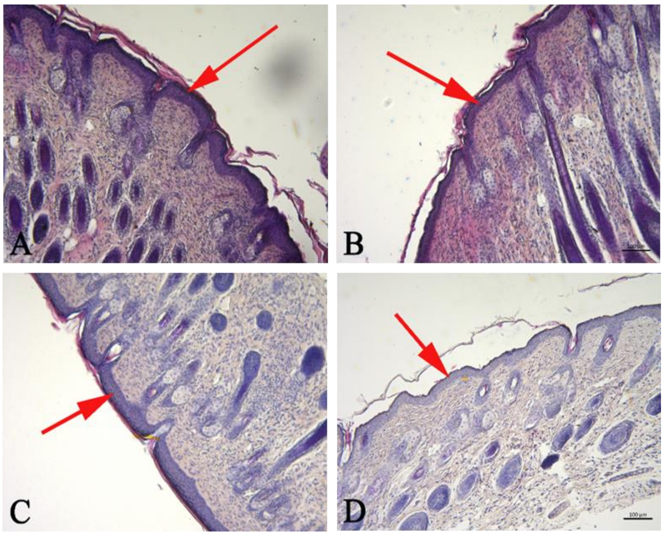

Figure 3: Histological results of wound tissue staining using Hematoxylin Eosin on the 15th day of each group (A: PCO (group 1); B: PCO1 (group 2); C: PCO2 (group 3); D: lanolin as control (group 4))

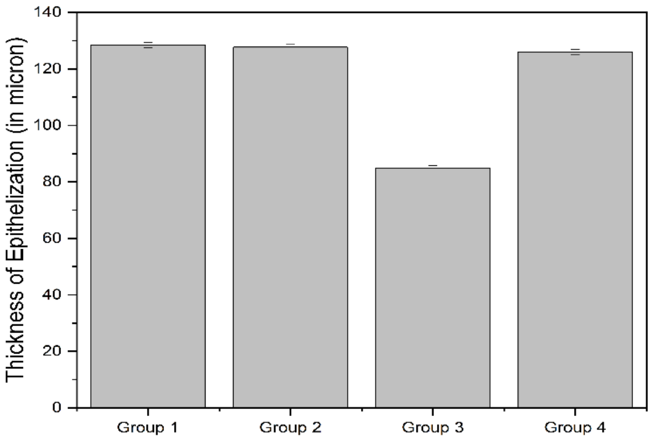

Figure 4: The average amount of epithelialization for each treatment groups. Group 1 (PCO), Group 2 (PCO1), Group (3 (PCO2), and Group 4 (lanolin)

Conclusion

In conclusion, the purification of CPO produced 2 types of oil, namely light and heavy fractions. The 3 types of patchouli oil showed good quality according to the characterization test and GC-MS analysis. Furthermore, the effect of crude oil, light fraction, and heavy fraction patchouli oil on epithelialization in full-thickness wound in male mice was investigated in this study. The results showed an increase in epithelialization thickness in the groups treated with CPO and CPO1, while a decrease was observed in samples treated with CPO2. Although both crude and the light fraction of patchouli oil had a comparable ability to enhance epithelial thickness in mice, the recommended choice for wound healing was light fraction. This was attributed to the lower impurities and iron content compared to crude variant. This study also showed the prospective application of light fraction of patchouli oil as a future study focus for the potential in wound healing.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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