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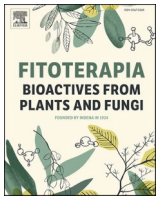

Research Paper

Accelerated wound healing with topical formulation based on *Lobularia maritima* essential oil: A rat model study

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ABSTRACT

This study investigated the wound-healing effect of the essential oil of *Lobularia maritima* (*Lm*EO) in a rat model. The animals were divided into three groups ($n = 8$): control (physiological saline), positive control (Centella cream), and *Lm*EO cream treatment. The wound closure rates were measured over 14 days, and the healed wounds were histologically analyzed. The hydroxyproline and C-reactive protein (CRP) levels were analyzed in the blood and tissue. The topical application of *Lm*EO significantly improved wound healing and reduced the inflammatory process compared to the negative and positive control groups. On day 14, the *Lm*EO-treated group achieved a wound contraction of 97 % ± 0.74 %, which was significantly higher than the control group (78.24 % ± 2.44 %) and the Centella cream group (85.62 % ± 1.68 %) ($p < 0.05$). The inflammatory protein content CRP was significantly lower in the group treated with *Lm*EO, namely by 51.96 % compared to the negative control group and by 34.28 % compared to the Centella cream group ($p < 0.0001$). Histopathological analysis further supported improved re-epithelialization, organized collagen and fibroblasts, and reduced inflammatory cell infiltration with *Lm*EO treatment. In conclusion, *Lm*EO, rich in bioactive oxygenated monoterpenes (>74.40 %), an effective agent for wound healing of the skin.

1. Introduction

The skin, as the body's largest organ, fulfills vital functions such as hydration, protection from external influences, synthesizing vitamin D, excretion, and regulating temperature. Serious skin injuries can be life-threatening and underline the crucial importance of effective wound healing [1,2]. Wound healing involves a series of phases including hemostasis, inflammation, proliferation, and remodeling [1]. Histological evaluation helps identify cellular changes specific to each stage. The skin, composed of the epidermis, dermis, and hypodermis, responds to injury with keratinocytes in the basal layer playing a key role in epidermal repair. The proliferative phase is characterized by granulation tissue formation, reepithelialization, and neovascularization and can last for several weeks. Oxygen plays a significant role in wound healing, impacting inflammation, bactericidal activity, angiogenesis, and epithelization. The process involves a complex interplay of cytokines and various cell types [3]. Hemostasis, the initial phase, typically occurs within minutes. Histological grading of wounds is important for assessing the different parameters of each healing phase [4,5] However, therapeutic constraints, particularly in treating chronic wounds, often present challenges in restoring tissue integrity. Consequently, research efforts are increasingly focused on developing more effective wound

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therapies to minimize healthcare costs and promote scarless healing [1,2,6].

In this context, the use of natural products has shown promise in preventing and treating wounds. Plants offer a broad spectrum of bioactive phytochemicals, including terpenes, which have been extensively studied for their diverse effects, such as antibacterial, anti-inflammatory, and analgesic properties, playing a central role in wound healing [7–11]. *Lobularia maritima* (*L. maritima*), a widespread halophytic ornamental plant on the Tunisian coast, is still relatively unexplored regarding its phytochemical composition. Studies have focused on characterizing the chemical composition of the different parts of the plant [8,12,13]. However, recent evidence suggests that L. *maritima* has potential iron-chelating properties and may protect against oxidative liver damage [13–15]. This protective property is thought to be due to the synergistic or individual action of tannins and flavonoids, known for their antioxidant properties. Phenolic compounds, which are widely present in L. *maritima* due to their high content of polyphenols, polysaccharides, and essential oils, have attracted attention due to their multiple biological effects [16,17]. The phytochemical composition of *Lobularia maritima* aerial part investigation showed the existence of some important flavonoids [18]. Moreover, the aerial parts' essential oil composition evidenced the presence of numerous oxygenated monoterpenes and monoterpene hydrocarbons such as linalool, terpinene, α-terpineol and α-pinene [16,19].

Despite its potential health benefits, *L. maritima* essential oil (*Lm*EO) has not yet been extensively researched for wound healing applications. Therefore, this work constitutes the first investigation dealing with the chemical identification of the bioactive compounds from L. *maritima* essential oil using gas chromatography–mass spectrometry (GC–MS) and proving its healing activity on a rat model.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents such as ethanol, NaOH, CuSO4, H2O2, and Ketamine /xylazine hydrochloride solution were purchased from Sigma Aldrich. CYTOL CENTELLA Cream from Cytolnat Laboratory (dermatological cream with *centella asiatica* extract to soothe and repair the skin) is used as a positive control for the wound healing study. An ELISA kit for CRP was purchased from (CRP LATEX, Biomaghreb).

2.2. Plant collection and authentication

L. maritima was collected from the Chebba region (Mahdia, North: 35.14° and East: 11.07°) in February 2021 and underwent recent studies to confirm its identity and authenticity, according to the Tunisian Flora. Post-harvesting, the aerial parts were dried at room temperature for two days in darkness.

2.3. Extraction of L. maritima essential oil (LmEO)

The essential oil (EO) extraction began with 1.2 kg of finely powdered plant components and 4 L of distilled water. The hydrodistillation took four hours and was carried out on a Clevenger apparatus. The aqueous phase was extracted with hexane (6 × 50 mL) and dried with anhydrous sodium sulfate to eliminate any remaining moisture. The solvent was subsequently removed by distillation at decreased pressure with a rotary evaporator. The pure oil was then properly kept at 4 °C in the dark until the analysis began. The yield of the extracted essential oil was determined according to the method previously described by Ben Akacha et al. (2022).

2.4. Preparation of L. maritima essential oil ointment and selection of positive control

The ointment of *Lm*EO was prepared according to Andjić's et al. (2021) protocol. The ointment was prepared by mixing 5 g of *Lm*EO with Eucerin ointment base until a homogenized sample was obtained. The Eucerin base contained a mixture of cholesterol (5 g), lanolin (15 g), paraffinum liquidum (15 g) and vaselinum album (65 g). The 0.5 % concentration of essential oil in gel and ointment was chosen based on literature data and relevant recommendations. Studies suggest that a dose of 5 % (*w/w*) appears suitable for topical application, while increasing the dose is not recommended due to the potential for skin irritation.

A commercially available Centella cream was used as the positive control. This product contains active compounds from *Centella asiatica* (asiaticoside, madecassic acid, and asiatic acid), which are widely recognized for their wound healing properties, including collagen synthesis stimulation, angiogenesis promotion, and enhanced re-epithelialization [20]. The full formulation also includes several plant-derived oils (grape seed oil, avocado oil, soybean oil, peanut oil) and skin-repairing agents like allantoin, tocopherol, and retinyl palmitate, making it comparable to other commercial phytotherapy-based wound healing products and suitable for comparison with our essential oil-based formulation [20].

2.5. Test no. 404: Acute dermal irritation/corrosion

The study was conducted following the Organization for Economic Co-operation and Development (OECD) guideline No. 404 of April 24, 2002. The principle of the test consists of applying the product to the epidermis of laboratory animals to evaluate its ability to cause skin irritation. The ointment was applied in a single dose to the shaved skin of rats; untreated skin areas of the test animal serve as the control. The degree of irritation/corrosion is read and scored at specified intervals and further described to provide a complete evaluation of the effects. Skin irritation was marked and recorded according to a scale of values described in OECD guideline No. 404 (2002) (from 0 to 4) (Table 1). The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects observed.

2.6. Animals and study design

Eighteen Wistar male rats weighing between 200 and 220 g were obtained from the Tunisian Central Pharmacy (SIPHAT, Tunisia). The experiment followed the guidelines of the Medical Ethics Committee for the Care and Use of Laboratory Animals at the Pasteur Institute in Tunis, Tunisia (approval number: FST/LNFP/Pro 152012), ensuring compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe No. 123, Strasbourg, 1985). The rats were given a 15-day acclimatization period in their new environment with free access to food and water [21]. Surgical procedures were performed after anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg i.p.), making an oval incision (1.5 × 1 cm) in the cervical area. The resulting wound was left untreated with antimicrobial drugs. To avoid any contact, each

Table 1
The skin irritation scale described in OECD Guideline No. 404 (2002).

Scores	Erythema
0	Complete absence of redness
1	Slight redness, just visible
2	Light red
3	Dark red, by zone
4	Dark red, very large

rat was individually kept in separate cages following surgery. The rats were then randomly separated into three groups, each with eight animals (Fig. 1). For 14 days, one daily topical application of 0.5 g of centella cream and *Lm*EO formulated cream. Following the methodology outlined by Demilew et al. (2018), the percentage of wound contraction was calculated on days 0, 2, 4, 6, 8, 10, 12 and 14.

$$\text{Wound closure}\ (\%) = [\text{Wound area day}\ (0) - \text{Wound area day}\ (n)\,]/\text{Wound area day}\ (0) \times 100.$$

2.6.1. Sample processing

Blood was taken from the brachial artery using heparin tubes and centrifuged at 2300 x*g* for 15 min to get plasma. At the same time, full-thickness skin samples from the wound area's dermal, epidermal, and subcutaneous layers were collected and delicately dissected. Preservation of both plasma and wound skin samples was carefully performed at −80 °C to ensure their integrity for future analytical studies [22,23].

2.6.2. Measurement of C-reactive protein (CRP)

The CRP value was determined according to the method described by Wu et al. (2002), Serum samples were analyzed using the CRP-LATEX ELISA kit (Biomaghreb) according to the manufacturer's guidelines for direct ELISA. Serum dilutions were prepared using a NaCl solution with a concentration of 8.5 g/L. Each serum dilution (50 μL) was mixed with well-homogenized anti-CRP latex (50 μL) on the card, stirred, and spread evenly. The card was rotated, and a possible appearance of agglutination was observed after 3 min. To estimate the CRP concentration in serum, the concentration of the last dilution that showed agglutination was multiplied by the sensitivity threshold of the test, which is 6 mg/mL.

2.6.3. Assessment of hydroxyproline

The collagen content in cutaneous wound samples was quantitatively estimated using a biochemical hydroxyproline assay, adapted from the method by Caetano et al. (2016) with specific modifications. The cutaneous wound samples were dried at 60 °C for 15 h. Then, 80 mg of dry samples were hydrolyzed using 8 ml of 6 N HCl at 130 °C for 4 h. Briefly, 0.3 mL of hydrolysate, 1 mL of 2.5 N NaOH, 1 mL of 0.01 M CuSO4, and 1 mL of 6 % of H_2O_2 were mixed and hooted at 80 °C. After 5 min, the samples were cooled for 10 min. Then, 0.6 mL of 5 % para-dimethyl amino-benzaldehyde and 1.2 mL of 3 N H_2SO_4 were added. The mixture was put in a bain-marie at 75 °C for 5 min. Finally, the optical density of the samples was measured at 540 nm. The hydroxyproline content of cutaneous wound samples was expressed as mg/g of dry tissue.

2.6.4. Histological assessment

To conduct histopathological analysis, fresh wound tissues were fixed in formalin solution (10 %) for 24 h, dehydrated in toluene and alcohol, embedded in paraffin, and sectioned at a thickness of 5 μm. Hematoxylin and eosin were then used to stain the sections. Micromorphological changes were examined using an Olympus BX51TF microscope [23] and graded with respect to epidermal regeneration, granulation tissue formation, inflammatory cell infiltration, angiogenesis and migration of fibroblast cells [24].

2.7. Statistical evaluation

The data was presented as the average ($n = 6$) ± standard deviation (SD). Statistical assessments were conducted using one-way ANOVA, followed by Tukey's *post hoc* examination for multiple comparisons. Significance among mean values was established at *p*-values of <0.05, <0.01, <0.001, and <0.0001.

3. Results

3.1. Safety assessment: Non-irritating profile of LmEO cream

According to the scale of values described in OECD Guideline No. 404 (2002), *Lm*EO formulated cream did not cause skin irritation on rats' dorsal part following application (Fig. 2).

Group 1: Group treated with physiological serum

Group 2 : Group treated with Centella cream

Groupe 3: Groupe treated with cream based on *Lm*EO at 5% cream

Fig. 1. Effect of various treatments on experimental groups: (1) control, (2) centella cream, and (3) *Lm*EO at 5 %.

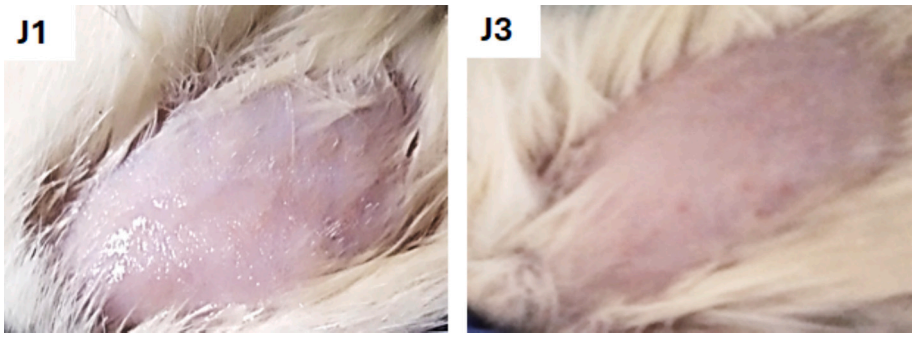

Fig. 2. Photographs of rats' dorsal areas following topical application of *Lm*EO formulated cream.

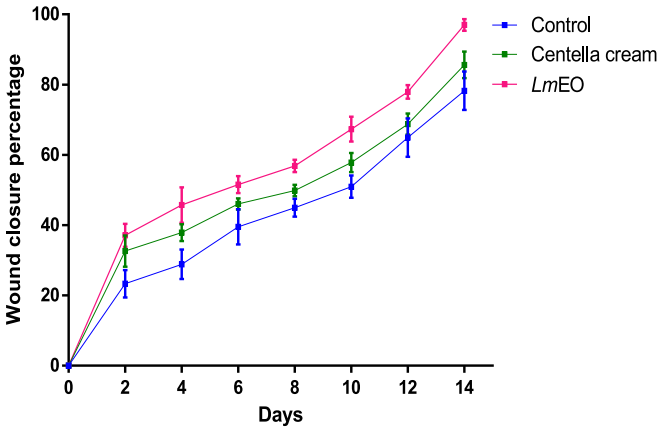

Fig. 3. Effect of Lm*EO* on wound healing expressed as a percentage of wound closure. Control (negative control) and Centella cream (positive control). Values are figured as mean ± SD ($n = 6$).

3.2. Accelerated wound contraction facilitated by LmEO cream

Wound contraction is described as the natural process by which the wound edges move closer throughout the healing process, whereas wound contraction is the shrinking of the scar itself. In this investigation, the percentage of wound contraction was measured throughout a 14-day trial period to assess the possible influence of *Lm*EO (5 %) on wound healing. Figs. 3 and 4 show the wound closure percentage and a photomicrograph of the wound scars. In fact, on day 14, the wound contraction in the control group was 78.24 ± 2.44 %, associated with a relatively large scar. However, for centella cream, we have obtained a significantly higher reduction in the wound area with 85.62 ± 1.68 %, whereas *Lm*EO formulated cream attends 97 ± 0.74 % ($p < 0.05$). From the 2nd day following the injury, which coincides with the beginning of the inflammatory phase in the healing process, a reduction in swelling of wounds treated with Centella cream and *Lm*EO was marked. By the end of the experiment, the whole area of the wound regained its original structure following the regular application of centella cream and *Lm*EO. Indeed, the best healing outcome was observed in the group treated with *Lm*EO, as shown by the wound closure percentages and the photographic representation of wound healing (Fig. 3 and Fig. 4). (See Fig. 5.)

3.3. Anti-inflammatory effects of LmEO: Reduction in CRP levels

C-reactive protein (CRP) is typically found in small amounts in serum under normal conditions, but it rises sharply in response to different infectious or inflammatory situations. In the present study, we have observed a reduction in the CRP levels in the group treated with Centella cream by 26.9 % and *Lm*EO by 51.96 % as compared to the negative control group ($p < 0.0001$). More interestingly, the topical application of *Lm*EO caused a significant reduction ($p < 0.0001$) in CRP levels by 34.28 % as compared to the centella cream application, indicating potent anti-inflammatory properties.

3.4. Enhanced collagen synthesis indicated by increased hydroxyproline

Hydroxyproline, an amino acid that is crucial for collagen synthesis, plays a vital role in wound healing. The hydroxyproline levels after treatment with *Lm*EO and Centella cream are shown in Fig. 6. The results clearly indicate a marked increase in hydroxyproline synthesis upon treatment with *Lm*EO at a concentration of 5 %, which was statistically significant ($p < 0.0001$) compared to the negative control and the centella cream-treated groups. It is particularly noteworthy that hydroxyproline synthesis was stimulated approximately twice as much as in the negative control group.

3.5. LmEO promotes improved re-epithelialization, collagen organization, and reduced inflammation

In this study, epidermal regeneration, collagen formation, revascularization, fibroblast organization, and inflammatory cell infiltration in wound tissues were assessed using hematoxylin and eosin-stained sections. The micrographic sections of the wound on day 14 after the injury are shown in Fig. 7, and their histopathological data are summarized in Table 2. Histopathological results of the negative control group indicated that the incision site had not yet been completely restored, and the re-epithelization was imperfect. In addition, immature fibroblasts have developed in the dermis of these rats. The collagen fibers recently formed were unarranged with the presence of inflammatory cell infiltration. On the other hand, the centella cream-treated group (positive control) showed an essential rise in the re-epithelization of the wound site and the deposition of oriented collagen fibers. This finding confirms that the remodeling phase has been completed. Besides, in the group treated with *Lm*EO, lesions were perfectly re-epithelized, the epidermis was effectively regenerated, and the boundary layer between the epidermis and the dermis was clearly marked. Compared to the negative control group, collagen fibers ($p < 0.01$) and fibroblasts ($p < 0.0001$) were greatly organized. Additionally, vasodilation, which was observed in the negative control group, was extensively corrected. Furthermore, reduced inflammatory cells and dense connective tissue were observed.

4. Discussion

The process of wound healing is a highly orchestrated physiological response crucial for restoring tissue integrity after injury, involving a precise cascade of biochemical and cellular events. The growing interest in natural products for wound prevention and treatment stems from their diverse bioactive phytochemicals, including alkaloids, carotenoids, phenolic compounds, steroids, flavonoids, saponins, tannins, and terpenoids [25]. Among these, terpenes are particularly noteworthy. Terpenes, extensively studied for their diverse effects, encompassing

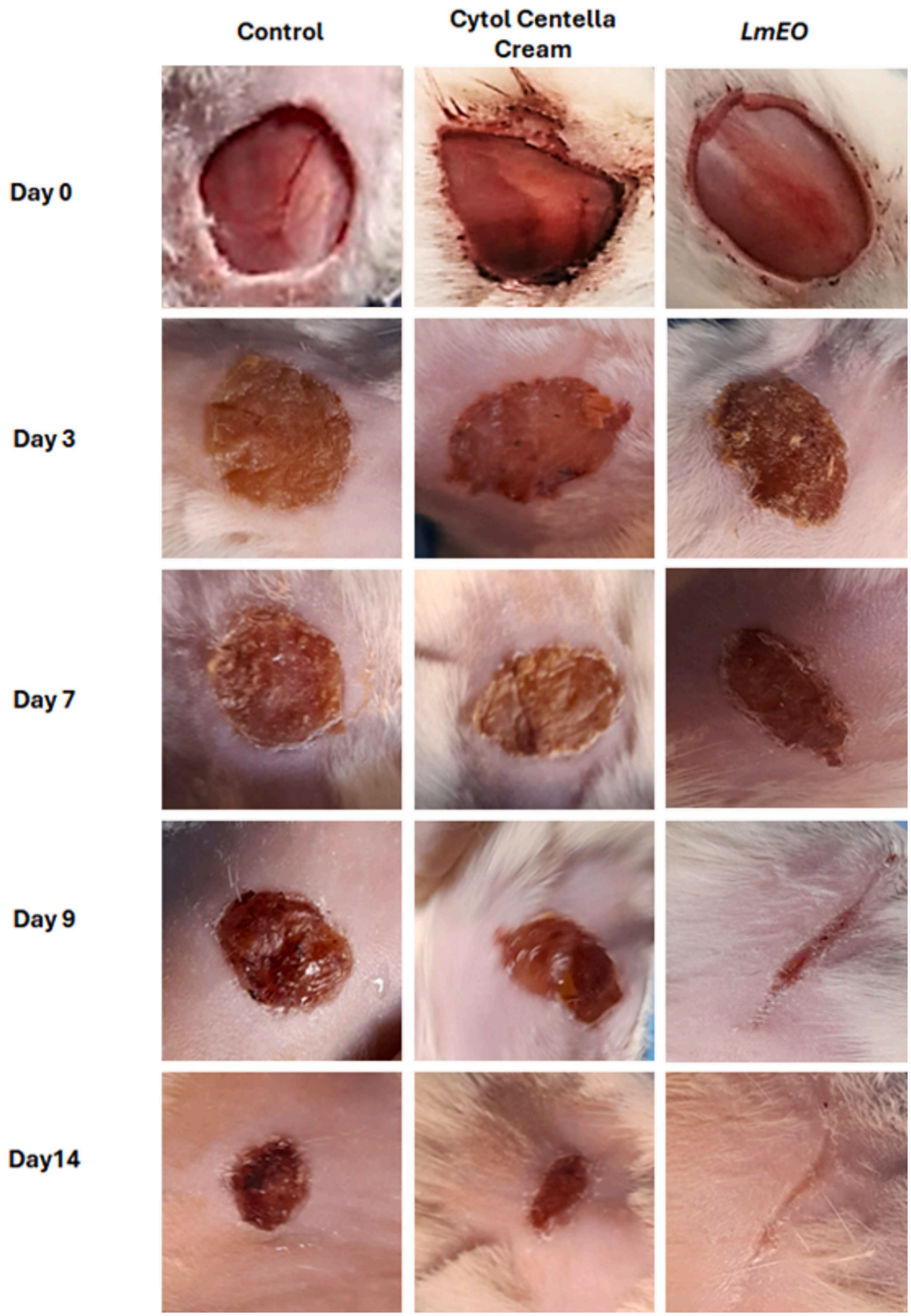

Fig. 4. Visual progression of healing: photographic documentation on days 0, 3, 7, 9, and 14 following wounding comparative evaluation of negative control (physiological serum), positive control (cytol centella cream), and *Lm*EO treatments.

bactericidal, fungicidal, insecticidal, anticarcinogenic, antioxidant, anti-inflammatory, analgesic, and sedative properties [26], play a central role in wound healing, often by acting as potent antibacterial and anti-inflammatory agents. For this reason, we sought to evaluate the potential of *Lm*EO to promote wound healing in a rat model. The chemical composition of *Lm*EO was characterized by GC–MS/MS analysis. *Lm*EO revealed 40 constituents with the main components included linalool (22.43 %), benzyl alcohol (8.65 %), 1-phenylbutanone (7.33 %), α-cadinol (4.91 %), globulol (4.32 %), α-terpineol (3.9 %), ledol (3.59 %), α-pinene (3.51 %), and pulegone (3.33 %). Similarly, a study conducted in China on L. *maritima* essential oil revealed about 40 compounds, with azeleonitrile (39.7 %), *trans*-3-pentenenitrile (36.3 %) and 4-isothiocyanato-1-butene (10.9 %) being the most abundant [27].

The wound healing test was performed using a formulated cream

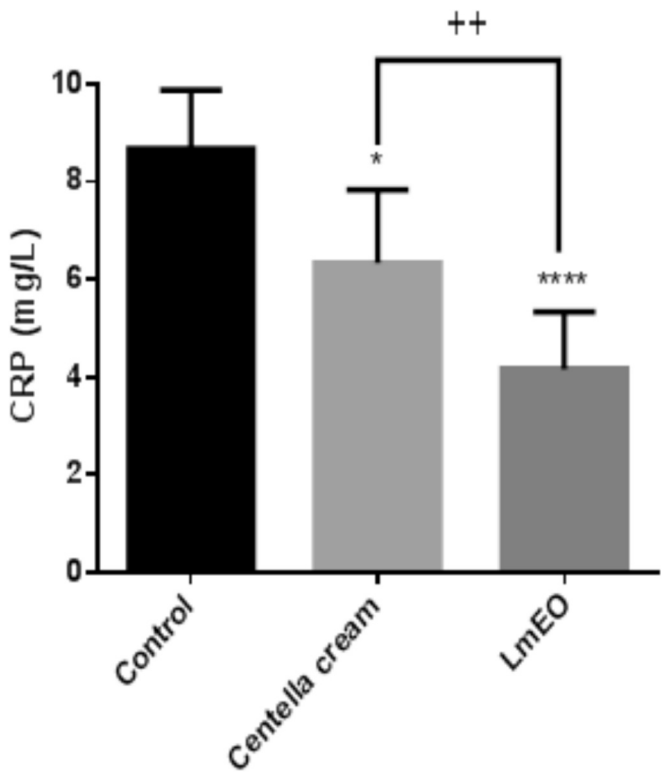

Fig. 5. Effect of *Lm*EO on CRP plasma levels. Values are represented as mean ± SD ($n = 6$). Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. *: $p < 0.05$, ****: $p < 0.0001$; significantly different compared to the negative control group. ++: $p < 0.001$; significantly different compared to the positive control group.

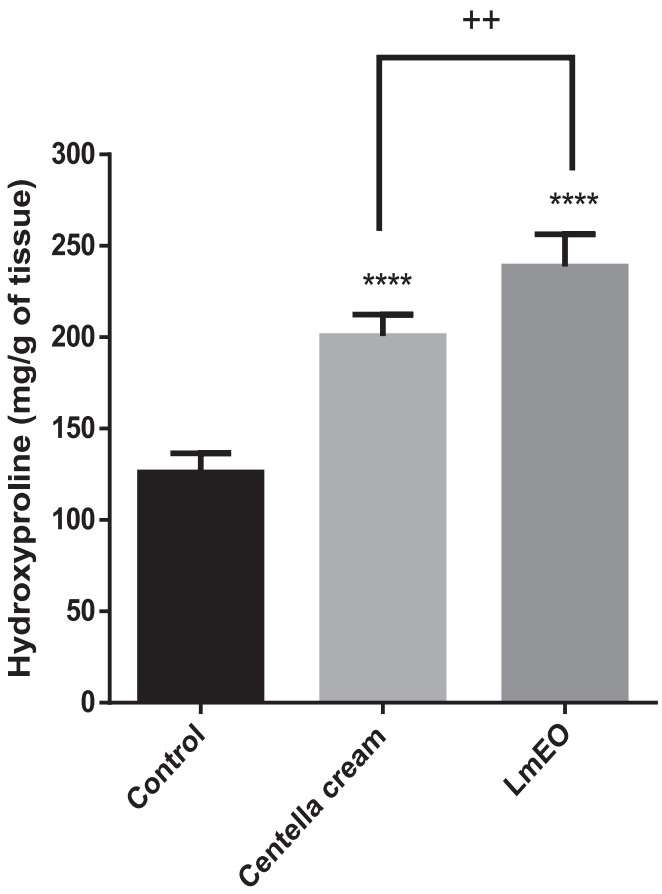

Fig. 6. Effect of *Lm*EO on hydroxyproline levels. Values are represented as mean ± SD ($n = 6$). Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. ****: $p < 0.0001$; significantly different compared to the negative control group. ++: $p < 0.001$; significantly different compared to the positive control group.

based on *Lm*EO. The ointment was tested first for acute dermal irritation. Our results unequivocally demonstrated that the *Lm*EO-formulated cream did not induce any skin irritation on the dorsal part of rats following application, thereby confirming its suitability and safety for subsequent *in vivo* wound healing evaluation. In this way, we ensured the quality of the cream that was subsequently used for the wound-healing test. The effects of the samples on skin healing were evaluated by physicochemical parameters (percentage of wound closure), biochemical changes (C-reactive protein and hydroxyproline concentration), and histopathological examination. This study showed that the groups treated with LmEO-formulated cream (5 %) and centella cream had faster wound contraction than the negative control group. The best wound closure was observed in the *Lm*EO-treated group. Previous studies have shown that the rapid reduction in wound area is related to fibroblast proliferation and collagen regeneration, leading to re-epithelialization of the wound site [28].

In the current study, the collagen amount in the wound site was evaluated in all groups. A healing tissue synthesizes collagen, which is a constituent of growing cells. The concentration of hydroxyproline is a measure of the concentration of collagen [29,30]. Therefore, a higher concentration of hydroxyproline indicates a faster rate of healing wounds. Our findings highlighted that *Lm*EO topical application accelerates hydroxyproline accumulation and, therefore, collagen synthesis in wound tissue, leading to a swift recovery of wounds. *In vivo* tests showed that terpenes such as α-pinene and α-phellandrene can accelerate wound contraction due to collagen deposition from the early stages in wounds [31]. Similarly, several studies have shown that the increased collagen synthesis may be because of the presence of large amounts of terpenes in the essential oils of several plants [10,32,33]. Some essential oils have generated significant interest in wound treatment due to their anti-inflammatory activity [34,35]. Therefore, the application of anti-inflammatory agents could be a valuable approach to accelerate the wound healing process [36,37]. Our results showed a potential reduction of the inflammatory marker CRP by topical application of *Lm*EO. According to Ben Akacha et al. (2022), the composition of *Lm*EO consists of 74.40 % oxygenated monoterpenes and 24.13 % monoterpene hydrocarbons. This composition makes *Lm*EO suitable for cosmetic applications and provides various benefits to the skin, including anti-acne, anti-ageing, and skin-whitening effects, making it a valuable active ingredient in the cosmetics industry [38]. These results are in line with previous research on *Rosmarinus officinalis* and *Populus alba* EOs, which have shown anti-inflammatory effects in both acute and chronic inflammation models. In particular, the treatments led to a progressive decrease in inflammation, with C-reactive protein levels decreasing to 28. Many commercial creams used to treat wounds have anti-inflammatory and antiseptic properties. These properties are well studied in our previous data showing the antibacterial and anti-inflammatory activities of *Lm*EO [39,40].

The observed anti-inflammatory and collagen-promoting effects of *Lm*EO are consistent with and build on the mechanistic understanding of other well-documented essential oils used in wound healing. For example, camomile oil (*Matricaria recutita*), a widely recognized therapeutic agent, has shown significant anti-inflammatory properties due to its main constituents, particularly chamazulene and α-bisabolol [41,42]. These constituents are known to modulate inflammatory pathways by reducing the synthesis and release of pro-inflammatory mediators and attenuating oxidative stress in the wound microenvironment [43]. In addition, studies investigating the effects of camomile oil on tissue

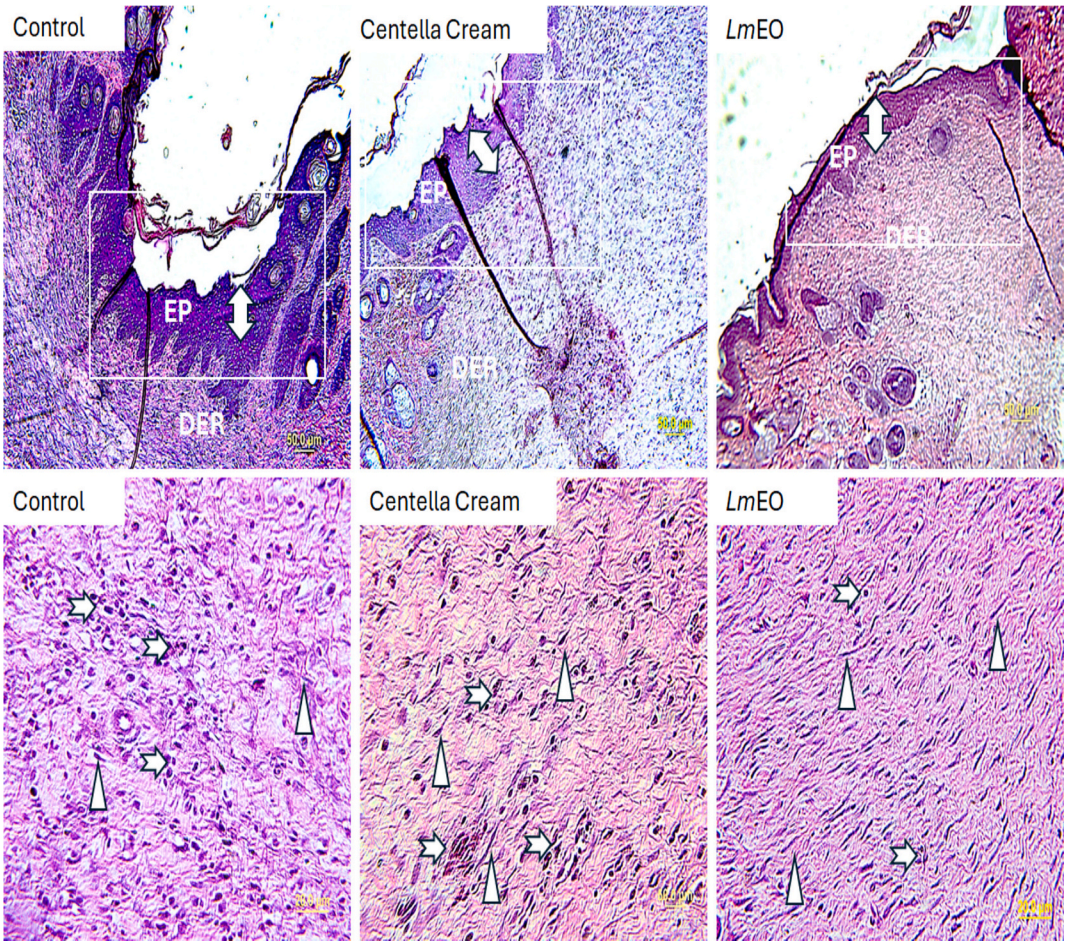

Fig. 7. Photomicrographs of the histological dermal section stained with hematoxylin-eosin dye at ×40 and ×100 magnifications, on day 12 post-wounding. Control (negative control), Centella cream (positive control), *Lm*EO formulated cream. EP: epidermis, DER: dermis. ⇨ Inflammatory cells; ◺ Collagen fiber; ⬚ Incision area

Table 2
Histopathologic scores evaluating of wound.

Feature graded	Grade	Description
Inflammatory infiltrate	1	Profound (>50 %)
	2	Scanty (10–50 %)
	3	A few (<10 %)
	4	Absen
Fibroblast proliferation	1	Mild
	2	Moderate
	3	Marked
Collagen formation	1	Mild
	2	Moderate
	3	Marked
New vessels	1	Mild
	2	Moderate
	3	Marked
Epithelium	1	Epithelial necrosis
	2	Epithelial proliferation on the edges of the ulcer
	3	Partial re-epithelialization
	4	Complete re-epithelialization
Epidermal differentiation	1	Basal cells
	2	Spinous epidermal differentiation (early)
	3	Granular epidermal differentiation (late)
	4	Complete

regeneration have consistently reported its ability to promote the formation of robust granulation tissue, accelerate re-epithelialization, and enhance collagen deposition, which overall contributes to more efficient and faster wound closure [44]. While both *Lm*EO and camomile oil exhibit potent healing properties through their anti-inflammatory and regenerative effects, the unique and diverse monoterpene profile of *Lm*EO, particularly the high concentrations of specific oxygenated monoterpenes such as linalool and α-terpineol, may offer distinct benefits or synergistic effects. This suggests that *Lm*EO is a valuable and potentially novel natural compound that enriches the therapeutic options for complex wound treatment, warranting further investigation of its precise molecular targets and comparative efficacy.

The histopathological study strongly corroborates our physicochemical and biochemical analyses, providing crucial morphological evidence. It indicates that *Lm*EO significantly improves wound closure by promoting complete re-epithelialization, facilitating the restoration of an organized dermal and epidermal structure, encouraging optimal rearrangement of collagen fibers and fibroblasts, and effectively reducing inflammatory cell infiltration. These comprehensive findings suggest the ability of *Lm*EO-formulated cream to actively stimulate healthy cells near the wound site, promoting their proliferation and the production of essential growth factors necessary for efficient healing and comprehensive skin regeneration.

5. Conclusions

Our study is the first to report on the wound-healing activities of *L. maritima* essential oil. Our findings showed that this EO contributed to the healing process by creating scars with high tensile strength and

accelerated wound closure through stimulating collagen deposition. The findings also revealed that *L. maritima* essential oil had a role in the healing process by promoting fibroblast migration to the wound site, which resulted in collagen formation. Furthermore, due to its anti-inflammatory and antibacterial properties, *Lm*EO could be recommended for treating small wounds, making it an essential active ingredient in the cosmetics industry.

CRediT authorship contribution statement

Boutheina Ben Akacha: Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Asma Mahmoudi:** Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Miroslava Kačániová:** Writing – review & editing, Validation, Supervision, Software, Investigation, Funding acquisition, Data curation. **Wirginia Kukula-Koch:** Writing – review & editing, Visualization, Supervision, Software, Investigation, Data curation. **Wojciech Koch:** Writing – review & editing, Visualization, Supervision, Software, Investigation, Data curation. **Rim Marrekchi:** Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Mohamed Chamkha:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation. **Rania Ben Saad:** Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Faical Brini:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation. **Wissem Mnif:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Data curation. **Stefania Garzoli:** Writing – review & editing, Visualization, Supervision, Software, Investigation, Data curation. **Anis Ben Hsouna:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Institutional Review Board Statement

The experiment followed the guidelines of the Medical Ethics Committee for the Care and Use of Laboratory Animals at the Pasteur Institute in Tunis, Tunisia (approval number: FST/LNFP/Pro 152012), ensuring compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe No. 123, Strasbourg, 1985).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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