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Literature Review

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Enhancing wound healing efficiency with fractionated *Schinus molle* essential oils

Abstract

The study focused on the extraction of essential oils from *Schinus molle L.* seeds using a novel pulsed steam distillation method, followed by fractionation to create an anti-inflammatory topical pharmaceutical product. This formulated product was unexpectedly employed in a specific case where an individual suffered a deep wound on the forehead due to an accident involving a sharp metal object, resulting in excessive bleeding. The oily product was directly applied to the wound, yielding remarkable outcomes. Notably, the bleeding stopped almost immediately, and the patient experienced an astonishingly rapid recovery, with complete closure of the wound observed within just five days. This remarkable healing time amounted to only a fifth of the expected duration for similar cases. The study highlights the significance of the extraction and fractionation processes, which were carried out using bench-scale devices, and underscores the importance of analyzing the chemical composition of the essential oils using gas chromatography coupled with mass spectrometry. These findings emphasize the critical role played by the pulsed steam distillation method and the formulation of the product in achieving exceptional wound healing performance.

Keywords: essential oils, *Schinus molle L.*, wound, enhancing, healing, pharmaceutical, ingredients, API

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Abbreviations: EO, essential oils; SML, *Schinus molle L.;* GC-MS, gas chromatography coupled with mass spectrometry; PSD, pulsed steam distillation; API, active pharmaceutical ingredient

Introduction

Essential oils are complex mixtures of odorous compounds found in various parts of plants or specific glands in animals. In plants, essential oils are synthesized in different organs such as leaves, bark, roots, flowers, stems, and even the entire plant, as seen in the case of eucalyptus.[1] Chemically, essential oils consist of a diverse array of species, including esters, ethers, aldehydes, ketones, alcohols, terpenes, sesquiterpenes, and more. These substances are hydrophobic, meaning they do not dissolve easily in water, and they are typically in liquid form, often lighter than wáter.[2]

One of the primary functions attributed to essential oils is their role in plant defense mechanisms. They are believed to protect plants from insects, microorganisms, and animals, acting as natural repellents or toxins. Additionally, some essential oils serve as attractants to insects, playing a crucial role in pollination. The use of essential oils by humans dates back to ancient times, with evidence of their registration dating back over 4000 years ago to the time of the pharaohs in ancient Egypt and even before.[3,4]

Throughout history, essential oils have played a significant role in various cultures due to their aromatic and medicinal properties. They have been used in rituals, perfumery, beauty treatments, and therapeutic applications, showcasing their versatility and widespread acceptance. The enduring significance of essential oils in human civilization is evident in their long-standing use and continued exploration.It is worth mentioning that there are several methods available to extract essential oils from plants, as can be seen in Figure 1 for brevity.

In perfumery, essential oils are highly regarded for their pleasant scents and their ability to create captivating fragrances. In medicine, specific essential oils are believed to possess antibacterial, antifungal, anti-inflammatory, or analgesic properties, making them valuable for therapeutic purposes. Essential oils also contribute unique flavors and aromas to food and beverages, and they hold potential for natural remedies and sustainable production methods in pharmacy and bioprocesses.[3]

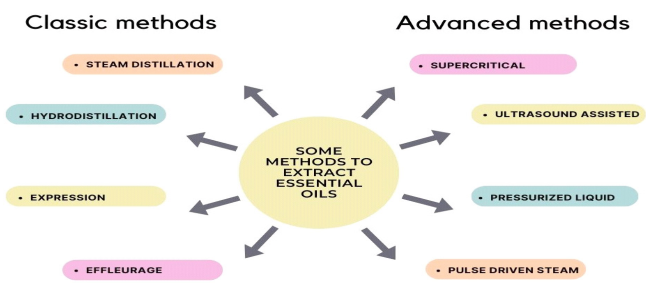

Figure 1 Some met hods to extract essential oils from plants.

In the context of this study, the essential oil obtained from *Schinus molle* L. seeds offers a unique combination of aromatic compounds that may have potential healing properties for wound treatment. By harnessing the knowledge and utilization of essential oils accumulated over centuries, researchers aim to explore their potential in developing innovative pharmaceutical formulations for enhanced wound healing.[5–7]

Literature review

Wounds are the result of a break in the skin surface, often caused by sharp objects or hard friction. Tissue injuries are repaired through the replacement of the damaged tissue in a period of time, ranging from days up to few weeks. If the tissue loss is minor, simply approximating the edges of the wound is usually sufficient. However, if there is significant tissue loss, the healing process can take much longer.[8]

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Wound healing is a complex process that has been extensively studied by researchers in various fields. Broughton et al.,[8] emphasized that our understanding of wound healing has evolved beyond the traditional three phases of inflammation, proliferation, and maturation. It now involves a multitude of reactions and interactions among cells and mediators. With each passing year, new mediators are discovered, expanding our knowledge of inflammatory mediators and cellular interactions. Broughton et al.,[8] further described the proliferative phase, which encompasses processes such as epithelization, angiogenesis, and provisional matrix formation occurring from day 4 to 14, while maturation and remodeling occur from day 8 to several months or even a year.

In order to promote optimal wound healing and minimize scarring, it is essential to consider various factors that can affect the healing process. Local wound care principles, as outlined by previous research[7], emphasize the importance of keeping the wound clean and moist, minimizing trauma, and preventing infection. Systemic factors, such as medications that may inhibit wound healing, proper nutrition, pain management, UV protection for the wound, and smoking cessation, also play a significant role in supporting effective wound healing.[6]

Regarding wound healing research, most scientists agree that the process is highly complex, involving various cell populations, the extracellular matrix, and the action of soluble mediators such as growth factors and cytokines. While healing is a continuous process, it can be arbitrarily divided into four phases: (i) coagulation and hemostasis, (ii) inflammation, (iii) proliferation, and (iv) wound remodeling, including scar tissue formation. These distinct phases contribute to the overall healing process and highlight the intricate mechanisms at play during tissue repair.[5–7]

The development of a pharmaceutical formulation involves the creation of a stable and patient-acceptable drug preparation.[8–10] Shabnam et al.,[11] highlighted the therapeutic applications of nanoparticles as delivery agents or broad-spectrum inhibition agents in viral and microbial investigations, demonstrating the potential of innovative approaches in wound healing research.

On the other hand, several studies have reported on the biological activity of essential oils, providing support for the healing properties of the fractions derived from *Schinus molle* sedes.[12,13] These findings suggest that essential oils may have the potential to enhance the wound healing process. Additionally, Ishida et al.,[14] conducted research on wound healing in diabetic mice and found that impaired healing could be reversed by topical administration of phyto pharmaceutical product, which restored neovascularization and macrophage infiltration.

In the realm of pharmaceutical research, Yang et al.,[15] explored deep learning methods for predicting pharmaceutical formulations. Their study focused on two model systems representing different dosage forms and evaluated the performance of the models using criteria specifically tailored for pharmaceutics. This research highlights the ongoing efforts to utilize advanced computational techniques in optimizing pharmaceutical formulations.

It is worth noting that the study of wound healing often presents challenges due to the substantial time required for comprehensive investigations.[16] Consequently, in-depth studies in this field are relatively rare. However, the importance of continued research in wound healing is evident in the pursuit of novel approaches to improve healing outcomes.

Moreover, natural antioxidants have garnered significant attention among professionals and consumers in the food industry due to their perceived safety and potential therapeutic value.[17] Their inclusion in wound healing research may offer additional benefits, considering the role of oxidative stress in the wound healing process.

The molle tree as a source of essential oils

Schinus molle L., commonly known as “molle” in Peru, is a native tree that grows extensively throughout the country. It held significant importance during the time of Wari´s and Incas, being utilized for various purposes including the production of beverages, construction materials, and the treatment of diverse ailments.

According to the report from the Natural History Museum of San Marcos University, *Schinus molle L.* falls under the following systematic classification: Division: Magnophyta, Class: Magnoliopsida, Subclass: Rosidae, Order: Sapindales, Family: Anacardiaceae, Genus: Schinus, Species: *Schinus molle* L.[18] The seeds of this plant form panicles, with small bright pink drupes measuring approximately 5 mm in diameter. These drupes are covered by two protective layers that can be mechanically removed through friction. When the seeds are well-cleaned, they exhibit a thin layer of sticky appearance consisting mainly of fermentable sugars, which can be leached with hot water to prepare a beverage known as “chicha,” still consumed in certain regions of the Peruvian highlands. Once dried, the seeds are crushed to undergo the isolation process in order to obtain essential oils. The resulting essential oil possesses a pleasant, somewhat resinous, and intense aroma.[18,19]

Materials and methods

Materials and methods are required in two separate fields, these being the separation process and pharmaceutical formulation. Therefore, in this work we consider it pertinent to state separately the requirements of both fields.

First of all, for the isolation of the essential oil.

Materials

1. *Schinus molle L.* seeds: These are the source of the essential oils to be extracted.
2. Steam generator: A device used to produce steam for the extraction process.
3. Extraction chamber: A container designed to hold the seeds and facilitate the extraction process.
4. Condenser: A component that cools and condenses the steam, allowing for the separation of the essential oil.
5. Collection vessel: A container used to collect the extracted essential oil.

Methods

1. Preparation of seeds: The *Schinus molle L.* seeds are cleaned and dried to remove impurities and moisture, ensuring optimal extraction conditions.
2. Loading the extraction chamber: The dried and crushed seeds are placed in the extraction chamber, which is then sealed to create a closed system.
3. Steam generation: The steam generator is activated to produce steam, which is then introduced into the extraction chamber. The steam carries the essential oil compounds away from the seeds.
4. Pulsed steam extraction: The steam is pulsed or intermittently applied to the seeds in a controlled manner. This pulsation helps to release the essential oil compounds more efficiently.[1]

5. Condensation: The steam, carrying the essential oil compounds, enters the condenser. The condenser cools the steam, causing it to condense back into liquid form. As a result, the essential oil separates from the water vapor.
6. Collection of essential oil: The condensed essential oil is collected in a separate vessel, separate from the water byproduct.

Separation and purification: Further purification processes is employed to separate any remaining impurities from the extracted essential oil, ensuring its purity and quality by then submitting the product to fractioning.

Now, to formulate a pharmaceutical product for injury healing, the following materials and methods are employed:

Materials

1. Essential oils: Essential oils derived from plants known for their wound healing properties, such as *Schinus molle L*.
2. Excipients: Various excipients are used to enhance the stability, solubility, and delivery of the essential oils.
3. Base or carrier materials: Suitable base or carrier materials are used to create the product's physical form.
4. Equipment: Mixing equipment, such as blenders or homogenizers are used.

Methods

I. Selection of essential oils: Essential oils with proven wound healing properties are chosen based on their chemical composition and therapeutic benefits.

II. Determination of concentrations: The appropriate concentration of each essential oil in the formulation is determined based on previous research, safety considerations, and desired therapeutic effects.

III. Selection of excipients: Excipients are carefully chosen to optimize the stability, solubility, and delivery of the essential oils. Compatibility between the essential oils and excipients is evaluated to ensure formulation effectiveness.

IV. Preparation of the base or carrier material: The base or carrier material, to established protocols or by following appropriate compounding techniques.

V. Mixing and blending: The essential oils and excipients are added to the base or carrier material using suitable mixing equipment. This ensures even distribution and uniformity throughout the formulation.

VI. Homogenization: Although advisable, we did not see the stability of essential oils and excipients afterwards.

Problem description

The problem of pharmaceutical product formulation for wound healing revolves around developing a stable and patient-friendly product that effectively accelerates the healing process. It requires careful consideration of various factors, including wound care principles, systemic support, formulation compatibility, and the utilization of bioactive ingredients with proven healing properties. By addressing these challenges, the formulation of an effective wound healing product can significantly improve patient outcomes and contribute to enhanced healthcare practices.

Some characteristics of the essential oil of *Shinus molle L.*

In this particular study, the focus was on *Schinus molle L*. (SML) as the raw material for essential oil extraction. Before subjecting the SML to the pulsed steam distillation method, the plant material underwent thorough botanical and physical characterization. This preliminary step ensured that the necessary information about the plant was obtained, enabling a comprehensive understanding of its characteristics and properties.

The essential oil derived from *Schinus molle L.* is a yellow-green liquid with a distinctive and persistent odor. Its density is lower than that of water (0.875 g/cc). Based on our experience, the chemical constituents of the essential oil of *Schinus molle* L. can be classified into low, medium, and high molecular weight compounds.[1,2]

While the use of essential oils for wound healing, particularly in the formulation described in this work, is relatively recent, it is important to mention previous studies such as Saporito et al.,[13] who utilized nanoparticles as carriers for essential oils. Overall, the unique properties of *SML*. Essential oil, its chemical composition, and its historical usage provide a foundation for exploring its potential applications in wound healing. The formulation developed in this study aims to harness the healing properties of the essential oil, potentially offering a novel approach to wound management.

Experimental procedure for extraction of essential oils

The isolation process began with the cleaning and crushing of the SML seeds to an average size of approximately 3 mm in diameter. This size reduction step proved crucial in enhancing the subsequent extraction process. The novel steam pulsed distillation method was employed, utilizing pulsed steam as the driving force to extract the essential oils from the seeds. This innovative approach significantly increased both the rate of oil isolation and the overall efficiency of the distillation process.[20]

A notable advantage of the pulsed steam distillation method was its remarkable reduction in processing time compared to conventional steam stripping methods. The processing cycle was shortened from approximately 90 minutes to just 10 minutes. This dramatic time reduction was achieved without compromising the quality of the extracted essential oils. Furthermore, the pulsed steam distillation method demonstrated an enhanced ability to quantitatively extract a greater amount of the available essential oil constituents.

To gain insights into the chemical composition of the extracted essential oils, mass spectrometry coupled with gas chromatography (GC-MS) was employed. This analytical technique allowed for the identification and quantification of the various components present in the essential oils. The comprehensive chemical analysis provided valuable information about the specific constituents and their concentrations, contributing to a deeper understanding of the properties and potential applications of the essential oils.

GC-MS Analysis of the essential oil

The essential oil obtained through the novel isolation method described earlier was subjected to characterization using GC-MS analysis at the Biotec CMC Tumbes analytical laboratory in Peru. To ensure accurate results and minimize interference from potential isomers, GC-TRIPLE TOF instrumentation was employed.

The analysis results, presented in Table 1, revealed the identification of 36 chemical species, including some unidentified compounds grouped together as one entry, accounting for approximately 8.4% of the total composition. It is worth noting that the first 11 species accounted for 85.5% of the total weight of the isolated oil. Among these major components, alpha phellandrene emerged as the predominant compound, constituting approximately 30% of the overall weight of the essential oil.[21,22]

Table 1 GC-MS analysis of the essential oils of *Schinus molle L.* (1)

Nº	Chemical species	%	Nº	Chemical species	%
1	Alpha –Phellandrene	30,00	20	Tricyclene	0,02
2	Sabinene	12,00	21	Alloaromadandrene	0,27
3	Limonene	10,00	22	Eremophylene	0,24
4	Para –Cymene	9,50	23	Aromadandrene	0,15
5	Beta- Phellandrene	8,00	24	Thujanol	0,14
6	Alpha- Pinene	5,00	25	Camphene	0,13
7	Terpinene 4 Ol 1	3,00	26	Copaene	0,13
8	Beta - Caryophylene	3,00	27	Gamma - Himachalene	0,13
9	Beta – Pinene	2,00	28	Acetate Bornyle	0,09
10	Gamma – Terpinene	1,50	29	Palustrol	0,09
11	Delta – Cadinene	1,50	30	Nerol	0,07
12	Alpha - Gurjumene	0,85	31	Carvacrol	0,07
13	Terpinene- Alpha	0,75	32	Citronellol	0,05
14	Alpha- Humulene	0,70	33	Alpha - Cubebene	0,04
15	Sabinene Trans Hydrate	0,55	34	Guaiarome	0,04
16	Germacrene	0,43	35	Gamma - Cadinene	0,04
17	Alpha - Muurolene	0,40	36	Ledene	0,03
18	Terpinolene	0,35	38	Unknowns	8,00
19	Gamma - Muurolene	0,34	39	Unknowns	0,40

(1)Data reported by Palomino.[18]

This analytical characterization provides valuable insights into the chemical composition of the essential oil isolated from *Schinus molle L.* seeds. The identification of various chemical species, particularly the significant presence of alpha phellandrene, contributes to our understanding of the potential therapeutic properties and applications of the isolated oil in the context of wound healing and other related fields.[23]

Formulation of the pharmaceutical product for injury healing

The formulation of the pharmaceutical product, firstly consist in extraction of essential oil from SML sedes under the action of pulsed steam, which is further fractionated to obtain more concentrated fractions.

Secondly, the pharmaceutical wound healing product is formulated following the steps described earlier.

Finally the efficacy of this product was tested in a case study, yielding unprecedented enhanced results, which will be shown in what follows.[1]

Application for topical treatment

After formulating the pharmaceutical product the following steps are advised to be taken in order to obtain the expected enhanced injury healing property.[20–22]

Application of the Formulation: The formulated product, consisting of the essential oil and excipient, is applied directly to the wound site. It can be administered using a sterile dropper, applicator, or by gently pouring the solution onto the wound, ensuring complete coverage.

Absorption and Action: The essential oil, along with the excipient, penetrates the wound bed and interacts with the damaged tissues, cells, and biochemical processes involved in the healing response. The active components of the essential oil facilitate the regeneration of skin cells, reduce inflammation, and promote wound closure.

Repeated Application: The treatment is repeated at regular intervals, typically as directed by a healthcare professional or as indicated on the product packaging. This ensures a continuous supply of the essential oil and excipient to the wound, supporting the healing process over time.

Monitoring and Evaluation: The progress of wound healing is monitored regularly to assess the effectiveness of the treatment. This may involve visual inspection of the wound, measurement of wound size reduction, assessment of pain levels, and monitoring for any signs of infection or complications. Continued Treatment: The wound healing treatment is continued until the wound has completely healed. The duration of treatment may vary depending on the severity and nature of the wound. It is essential to follow the recommended treatment duration and any additional instructions provided by healthcare professionals.

The case study setting and observations

In this case study, we present the application of the formulated wound healing product in a real-life scenario. The incident occurred when the patient suffered an accidental injury by falling and hitting his forehead on a metal device. The incident took place at approximately 3 AM, and the patient immediately sought assistance. Since there was no other available option at that moment, the family assisting him decided to use the aforementioned wound healing product, which had been previously formulated for topical treatment of arthritis using natural ingredients (Figure 2).

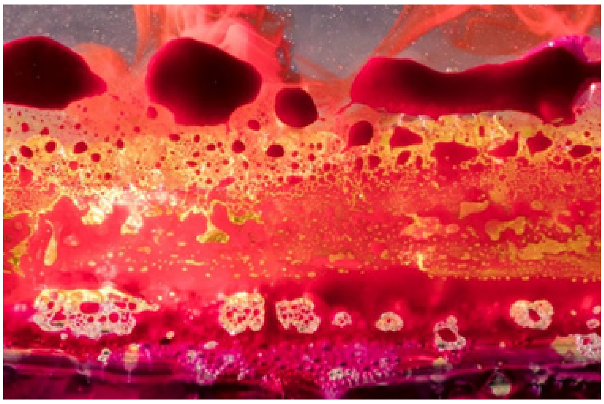

Figure 2 Wound image as taken from Wepik.[25]

The evidence of this case is the fact that the patient managed to recover from the injury in one-fifth of the expected time, even after undergoing a small surgical procedure. This outcome revealed the enhanced ability of the essential oil to promote the healing of skin wounds by accelerating the process of cell regeneration. As a result, the healing time was reduced to nearly one-fifth, with the overall healing period shortened from 25 days to approximately 5 days. The evidence suggests that both the isolation method and the formulation process played crucial roles in achieving such remarkable results. It is worth noting that the healing product has an oily appearance, and the case study involved an individual who suffered an accidental wound, which is reported among a group of five individuals.

The improvement in the pharmaceutical formulation of the wound healing product has been achieved by bringing chance and causality together.The updated formulation now incorporates a synergistic blend of active ingredients known for their wound healing properties.[24] These include natural extracts, that is essential oils of SML with antimicrobial, anti-inflammatory, and tissue-regenerating effects, combined with appropiate excipients to create a stable and effective pharmaceutical formulation. The excipient include a suitable base or carrier material that enhances the delivery and efficacy of the essential oil. We will stop releasing further details at this point for patenting reasons.

Photographical sequence of wound healing and observation

To provide visual evidence and facilitate the discussion on the healing process of the injury, we present the following photographic sequence. The images depict the different stages of the healing process, showcasing the remarkable transformation and progress observed.

Generally speaking, the treatment process involves cleaning the injured area with distilled water to ensure cleanliness and prevent infection. The formulated healing agent, containing essential oils and excipients, is then applied directly to the wound. This immediate application effectively stops bleeding and promotes the formation of blood clots, which act as a protective seal. The healing agent is reapplied every 6 hours for 5 to 6 days to maintain continuous healing and prevent complications. After the external dry clot naturally detaches, the application frequency is reduced to every 12 hours until the wound is completely healed. This pharmaceutical formulation of essential oils and excipients accelerates wound closure and reduces healing time. It is important to note that further studies and clinical trials are needed to validate the efficacy and safety of the product on a larger scale. Nonetheless, this case study provides valuable insights into the potential benefits of the formulated wound healing product and encourages further exploration in similar scenarios to improve patient outcomes and healthcare practices.[25]

Discussion

We deem worthy to remark that during the observation period, we noted a rapid cessation of the profuse bleeding after the application of the wound healing product. Subsequently, a clot formed on the surface of the injury, initiating the healing process. Over time, we observed the development of granulation tissue and a remarkable reduction in the swelling around the eyes, leading to their complete resolution within five days. It is worth mentioning that no keloidal tissue formation was observed, which is typically associated with prolonged healing. The healing progression appeared to be accelerated, resembling a microsurgery procedure. These findings highlight the exceptional efficacy of the wound healing product in promoting rapid and efficient wound closure without the formation of keloids (Figure 3).

Figure 3 Depicts of the observed healing stages in the patient, as follows:(0) Unfortunately, the photography was missed due to the need for immediate emergency assistance.(1) The healing progress observed one day after the accident.(2) and (3) The healing progress observed two days after the accident. (4) The healing progress observed three days after the accident.(5) The injury fully healed as observed six months later.(6) The patient's departure from Peru at the Peruvian airport after a visit with family.

Conclusion

The chosen pharmaceutical active ingredients for the formulation of the injury-healing product appear to be well-balanced and act synergistically as excipients, carriers, antiseptic agents, and cell bonders, facilitating rapid granulation and wound closure, which may be inferred from the evidence shown in Figure 3.

Remarkably, the healing time in the reported Case Study was only 5 days up to the granulation stage. This represents a significant reduction compared to the expected healing time of approximately 25 days for granulation completion, as stated in the literature. These results highlight the effectiveness of the novel extraction method, the well-chosen pharmaceutical active ingredients, and the potential for further investigation and optimization in the field of wound healing.

The novel steam pulsed method for isolating essential oil from *Schinus molle L.* seeds significantly reduces the extraction time from 90 minutes to just 10 minutes. We consider that this improved method has a direct impact on the quantity and quality of the essential oils used in formulating the injury-healing product, making it one of the key factors for enhancing the product.

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Conflicts of interest

We have no conflict of interest.

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