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ORIGINAL ARTICLE

Therapeutic Efficacy of Plant-Based Hydrogels in Burn Wound Healing: Focus on *Satureja Montana* L. and *Origanum Vulgare* L.

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Abstract

Background/Aim: Burn injuries present a critical issue for healthcare systems worldwide. They often lead to numerous complications, including persistent inflammation, impaired healing and heightened infection risks. The growing challenge of antibiotic resistance further complicates treatment, making bacterial infections harder to control and wound healing less effective. Existing treatment methods are often limited, highlighting the need for alternative approaches. Aim of this study was to analyse influence of hydrolate-based gels derived from *Satureja montana* L. and *Origanum vulgare* L. on the healing of burn wounds.

Methods: New Zealand white rabbits (n = 25) with standardised Grade IIIa thermal burns were assigned to treatment groups receiving hydrolate-based gels (*Satureja montana* L. or *Origanum vulgare* L.), conventional therapy (*Betadine* (povidone-iodine) and *Levomecol* (methyluracil, chloramphenicol)), or no treatment. Inflammatory responses were tracked by collecting blood samples at the study's begin, on day 3 and at one- and two-weeks post-burn, with subsequent ELISA analysis. Histological evaluation of tissue regeneration and inflammatory response was conducted using haematoxylin and eosin staining on days 3, 7 and 14.

Results: By day 14, *Satureja montana* L. demonstrated the most effective reduction in interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP) levels, nearing baseline values. This biochemical improvement correlated with histological findings, showing advanced epithelial regeneration, reduced inflammatory cell infiltration and clearer dermal-epidermal boundaries. The results highlight *Satureja montana* L. as the most effective treatment in both inflammation control and tissue repair compared to *Origanum vulgare* L. and conventional therapy.

Conclusion: Hydrolate-based gels from *Satureja montana* L. and *Origanum vulgare* L. represent a promising option for adjunctive burn wound therapy. The *Satureja montana* L. group demonstrated significant improvements in inflammation control and tissue regeneration, while *Origanum vulgare* L. showed results comparable to standard therapy.

Key words: Burns; *Satureja, montana* L.; *Origanum, vulgare* L.; Plant extracts; Inflammation; Regeneration.

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Introduction

Thermal injury represents a significant issue worldwide, causing approximately 180,000 fatalities annually.[1] These injuries initiate complex pathological processes, including extensive tissue

necrosis, persistent inflammation and increased susceptibility to infections.[2, 3] Such complications often result in delayed wound healing, prolonged recovery periods and increased risks of systemic infections and chronic pain.[4-6] Despite advancements in burn care, severe burns continue to result in substantial morbidity and mortality.

Therapies (antimicrobials and anti-inflammatory drugs) are often limited. The rise of multidrug-resistant bacteria complicates infection control, while delayed wound healing persists as a major challenge.[7] Furthermore, traditional therapies often target isolated aspects of burn wound pathology rather than addressing the interconnected processes simultaneously (for example, while antimicrobial agents can reduce infection risk, they may not effectively modulate inflammation or promote tissue regeneration; anti-inflammatory drugs may control excessive immune responses but fail to enhance cellular proliferation required for wound closure). These limitations highlight the urgent need for alternative therapeutic approaches capable of addressing these interconnected processes more effectively.

Phytotherapeutic agents derived from medicinal plants offer interesting alternatives for burn wound management due to their anti-inflammatory, antibacterial and oxidative stress-reducing capacity.[8] Among them, *Satureja montana* L. and *Origanum vulgare* L. are notable for their potent bioactive compounds, including phenolic acids, flavonoids and essential oils, which contribute to their therapeutic effects.[9, 10] Hydrolates, water-based extracts obtained via steam distillation, deliver these compounds in a form suitable for safe topical application.[11] Notably, the plants used in presented study were harvested from a region characterised by a unique combination of mountainous terrain and maritime climate. These ecological conditions are known to enhance the biosynthesis of bioactive compounds, potentially amplifying their therapeutic efficacy.[12, 13] Despite this, their application in burn wound healing remains insufficiently studied. Further investigation into the mechanisms underlying their effects on tissue repair and infection control is essential for advancing their clinical use.

In light of this, hypothesis was made that hydrolate-based gels derived from *Satureja montana* L. and *Origanum vulgare* L. – leveraging these unique ecological conditions – could accelerate burn wound healing by modulating inflammation and enhancing tissue regeneration. Aim of this study was to assess the regenerative effects of hydrolate-based gels on skin healing following burn injuries.

Methods

Experimental animals

Twenty-five male rabbits (White New Zealand; 3.0 kg ± 330 g; age: 7–8 weeks) were used. Each rabbit was individually housed in stainless steel cages compliant with animal welfare standards, ensuring adequate space (4 square feet, 20 inches in height) and hygiene, with soft bedding material provided for comfort (*Delta Feeds*, Moscow, Russia).

Male rabbits were used to avoid hormonal variability from the female oestrous cycle. Including female animals in future studies remains essential for broader translational relevance and a comprehensive understanding of therapeutic efficacy across genders.

Experimental design

Animals were randomly assigned to five groups to ensure unbiased allocation (Figure 1):

- Group I (n = 5): Intact control animals (without burn injury);
- Group II (n = 5): Animals with burn injury without treatment;
- Group III (n = 5): Animals with burn injury receiving conventional therapy (boric acid and gauze dressings soaked in *Betadine* (povidone-iodine) and *Levomecol* (methyluracil, chloramphenicol);
- Group IV (n = 5): Burn injury treated with gel prepared from *Satureja montana* L. hydrolate;
- Group V (n = 5): Burn injury treated with gel prepared from *Origanum vulgare* L. hydrolate.

Baseline assessments included weight measurements, behavioural reactions and biochemical analysis to exclude animals with physiological deviations. Blood from marginal ear (1–1.5 mL) vein at baseline (24 h before burn induction) was collected and on days 3, 7, 10 and 14 post injury, adhering to all institutional ethical standards for animal care.

Biopsy samples for histological analysis were gathered at all experimental time points. Necrot-

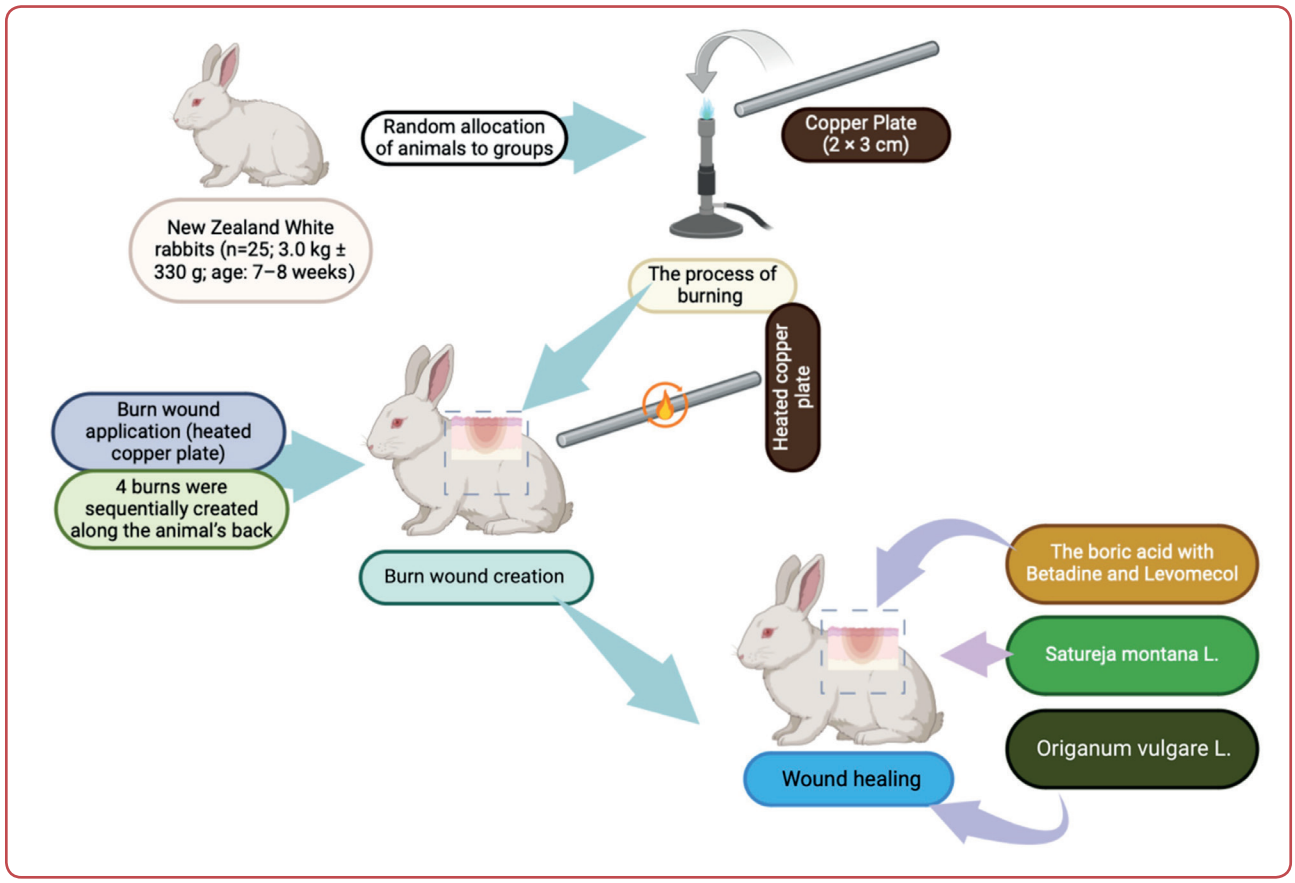


Figure 1: Schematic representation of the experimental design

ic tissue was excised using surgical tweezers, a scalpel and fine-point scissors, followed by wound cleaning with a 0.5 % chlorhexidine solution to minimise bacterial contamination. In cases of unresolvable complications, animals were humanely euthanised using Zoletil® 100 (tiletamine and zolazepam) (15 mg/kg body weight) and xylazine (5 mg/kg body weight), ensuring a painless and ethical procedure.

Burn injury induction and treatment protocol

Standardised grade IIIa thermal burns under neuroleptanalgesia (Zoletil® 100, *Virbac*, France; 5 mg/100 g body weight) were induced. A pre-heated copper plate (2 × 3 cm, 200 g) was applied with a force of 2 N for 30 se on the pre-shaved dorsal surface of each rabbit. Four identical burns were created sequentially along the back. Treatment began 24 h post-injury and was applied twice daily at 10-hour intervals using sterile cotton applicators. Animal health parameters, including behaviour, appetite, water intake and rectal temperature, were monitored throughout the study.

The severity of grade IIIa burns was confirmed using established criteria[14-16]: 1. Eschar formation: dense, leathery eschar indicating deep coagulative necrosis; 2. Loss of skin elasticity: stiff, non-pliable tissue reflecting collagen denaturation; 3. Pain response: absence of pain response to light touch, signifying nerve damage; 4. Colour change: white or charred appearance, indicating tissue coagulation; 5. Blister formation: delayed fluid-filled blisters, distinguishing from superficial burns.

Dosage form

Therapeutic gels were prepared from hydrolates of *Satureja montana* L. and *Origanum vulgare* L. via steam distillation of 500 mg fresh plant material. The hydrolates, clear to slightly tinted (pH 3.2–6.0). To achieve a uniform gel consistency, 2 % hydroxyethyl cellulose (HEC, 20 g/L) was added at room temperature with constant stirring, followed by gentle heating to 40 °C. The final gels were stored in sterile, airtight containers at 4 °C to maintain stability and microbial purity.

Biochemical assay

Serum samples were processed by centrifugation at 3000 rpm for 12 minutes (the storage temperature was −80 °C). Commercial ELISA kits were used: C-reactive protein (*Life Diagnostics*, West Chester, PA, USA), interleukins: IL-1β and IL-6 (*Bender MedSystems*, Vienna, Austria), tumour necrosis factor alpha (TNF-α) (*Assaypro*, St. Charles, MO, USA). All procedures adhered to the manufacturers' protocols (calculations of optical density at 450 nm).

Morphological assay

Biopsy samples from wound sites were collected on all time points. Tissue specimens were preserved in 10 % neutral-buffered formalin, followed by dehydration and paraffin embedding. Histological examination: thin sections (3 μm) were prepared and stained using haematoxylin and eosin (H&E).

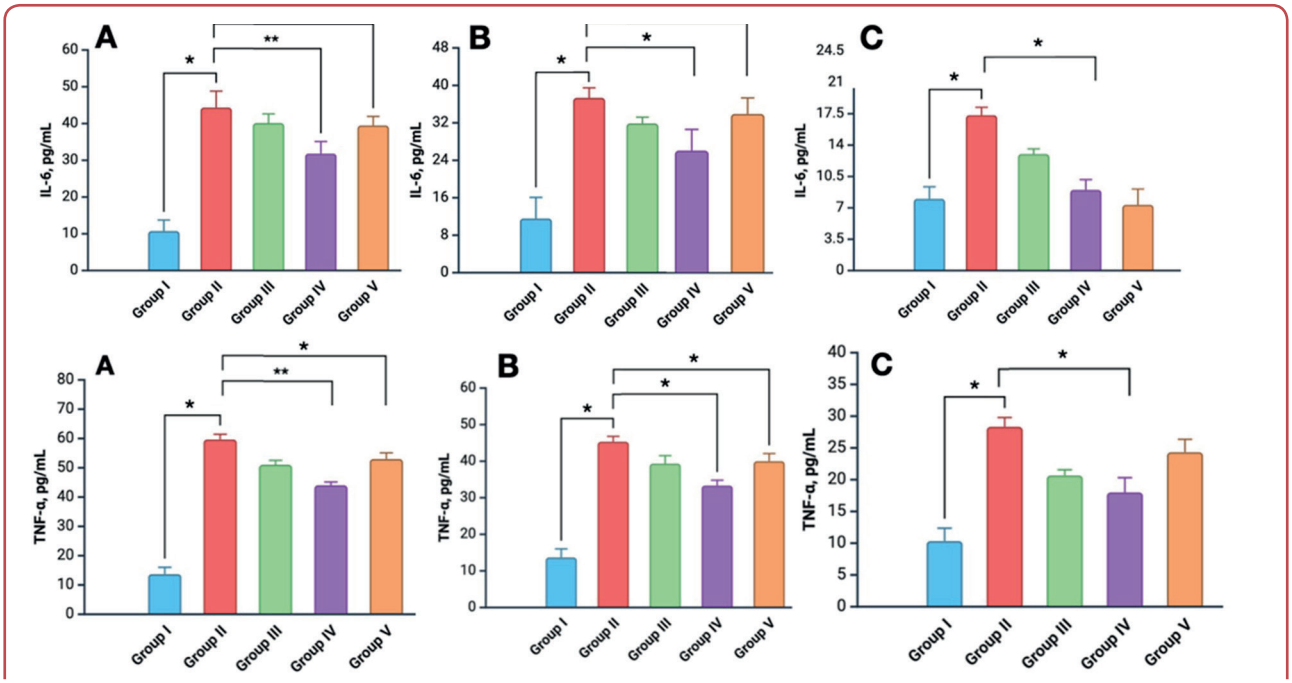
Statistical analysis

All statistical analyses were performed using SPSS 12 for Windows (*IBM Analytics*, Armonk, NY, USA). Data were presented as mean ± standard deviation (SD). The Shapiro-Wilk test assessed data distribution normality. Non-parametric comparisons across groups utilised the Kruskal-Wallis test with Dunn's post-hoc adjustment. Pairwise comparisons were conducted using the Mann-Whitney U-test, with significance set at $p \leq 0.05$.

Results

Biochemical assay

At baseline, all groups showed normal biochemical parameters, confirming no pre-existing inflammation or infection (Figure 2). On day 3, inflammatory markers peaked in the untreated group (Group II), reflecting an intense inflammatory response. Group IV (*Satureja montana* L.) demonstrated the most pronounced reduction in cytokine levels, followed by Group V (*Origanum vulgare* L.). Group III (*Betadine* and *Levomecol*) showed moderate improvements. By day 7, Group IV maintained the lowest cytokine and CRP levels, with Group V showing similar trends, while Group II remained elevated. On day 14, Group IV cytokine and CRP levels approached baseline, indicating near-complete resolution of inflammation. Group V also displayed significant improvement but remained slightly above baseline. Group II exhibited minimal changes, with persistently elevated marker levels.



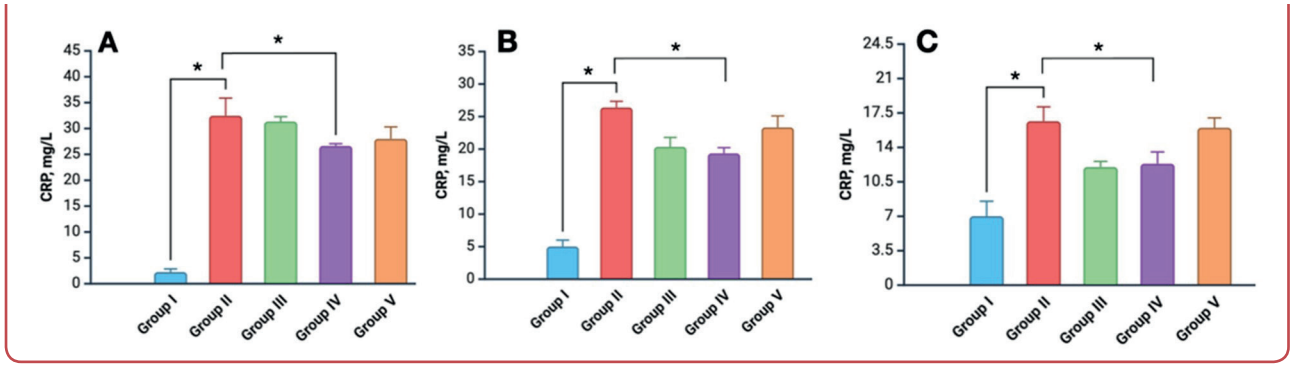
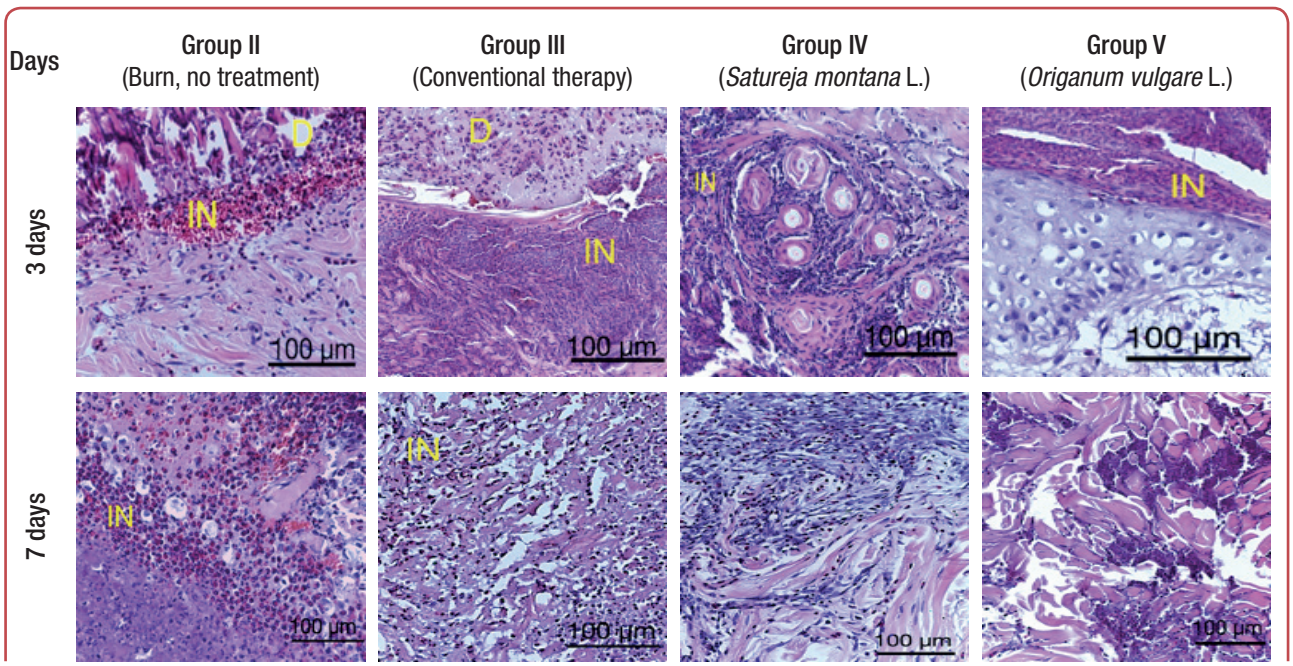


Figure 2: The temporal variations in serum levels of interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP)

*Experimental groups: Group I: Intact control animals (without burn injury); Group II: Animals with burn injury without treatment; Group III: Animals with burn injury receiving conventional therapy (boric acid and gauze dressings soaked in Betadine (povidone-iodine) and Levomecol (methyluracil, chloramphenicol); Group IV: Burn injury treated with gel prepared from Satureja montana L. hydrolate; Group V: Burn injury treated with gel prepared from Origanum vulgare L. hydrolate. Data are shown for three time points: A: 3 days post-injury, B: one-week post-injury and C: two weeks post-injury. Results are presented as mean ± SD. Statistically significant differences are marked using symbols: *p < 0.05 and ** p < 0.01.*

Morphological assay

Signs of a IIIa degree thermal burn in "no treatment group" at third day – serous inflammation was found, which was characterised by: destruction of the epidermis, the presence of cellular detritus on the surface, breakdown of the papillary region within the dermis, signs of moderate inflammatory infiltration of a diffuse nature, mainly segmented leukocytes, vascular reaction (Figure 3). The detected changes occupied almost two-thirds of the wound surface area. In dynamics, a gradual improvement in the morphological picture was noted; however, cellular detritus and individual neutrophils were still present two weeks post-injury. By day 7, Group IV (*Satureja montana* L.) exhibited the most advanced wound healing, with minimal exudate, reduced inflammation and initial signs of hair regrowth. Group V (*Origanum vulgare* L.) also showed notable healing but with a slight delay compared to Group IV. Group III (conventional therapy) demonstrated moderate improvements, with inflammation control but limited epithelial regeneration. Group II showed persistent purulent exudate, delayed healing and secondary infection signs. By day 14, Group IV achieved near-complete epidermal regeneration, minimal inflammation and improved tissue organisation. Group V displayed similar results. Group III exhibited moderate healing, while Group II showed structural disorganisation and limited epithelial repair.



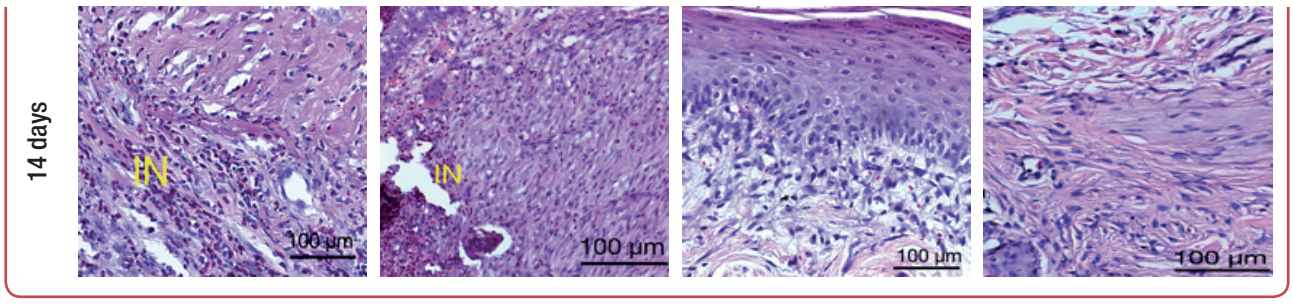


Figure 3: Representative histological images of burn wounds across experimental groups at 7- and 14-days post-injury, haematoxylin and eosin (H&E) staining, ×200 magnification

Conventional therapy – therapy with boric acid and gauze dressings soaked in Betadine (povidone-iodine) and Levomecol (methyluracil, chloramphenicol); D: detritus; IN: inflammation.

Discussion

This study evaluated the effects of hydrolate-based gels derived from *Satureja montana* L. and *Origanum vulgare* L. on burn wound healing, focusing on their capacity to regulate inflammation and promote tissue regeneration. Differences in healing dynamics, inflammation control and tissue recovery among the treatment groups were revealed, underscoring the therapeutic potential of these phytotherapeutic agents.

Burn injuries, affecting over 11 million people annually worldwide, remain among the most severe types of skin trauma.[17] They result from various causes, including thermal (exposure to high temperatures), chemical (eg, CH_3COOH, HCl), electrical and radiation sources.[18] The severity of burns, classified by tissue damage depth, determines treatment strategies, ranging from antimicrobial prophylaxis to surgical interventions.[19]

The initial damage caused by burns is frequently exacerbated by secondary complications, such as oxidative stress, excessive cytokine release and leukocyte infiltration, collectively intensifying tissue destruction.[3] Additionally, microvascular thrombosis and endothelial dysfunction impair blood flow, restricting oxygen and nutrient delivery essential for effective tissue repair.[4, 14] These complications prolong wound healing, increase susceptibility to infections and might provoke a systemic inflammatory response and sepsis, often resulting in multi-organ dysfunction and increased mortality.[6, 15]

The drawbacks such as side effects and the increasing prevalence of multidrug-resistant bacteria highlight the need for safer alternatives.[7] Hydrolates, also known as hydrosols, are water-based extracts obtained through the steam distillation of medicinal plants. Unlike essential oils, hydrolates contain water-soluble bioactive compounds, including flavonoids, phenolic acids and terpenoids, which exhibit potent antimicrobial, antioxidant and anti-inflammatory and other properties.[20] Their milder chemical composition compared to essential oils makes them particularly suitable for topical application, minimising risks of irritation or toxicity while preserving therapeutic efficacy.

Hydrolates of *Satureja montana* L. and *Origanum vulgare* L. are rich in rosmarinic acid and thymol, bioactive compounds with proven anti-inflammatory, antioxidant and antimicrobial properties.[21, 22] Their water-soluble nature ensures enhanced bioavailability and effective skin penetration, allowing them to act directly within the wound microenvironment. This combination of safety, therapeutic efficacy and bioavailability highlights hydrolates as promising alternatives to conventional burn treatments, particularly in controlling inflammation and preventing secondary infections. Further molecular investigations could optimise formulations and expand their clinical applications.

Inflammation plays a dual role in burn wound healing — it triggers tissue repair but, if prolonged, increases the risk of secondary infections.[23] In presented study, IL-6, TNF-α and CRP levels rose across all groups by day 3, reflecting an acute inflammatory response. CRP remained persistently elevated in the untreated group, indicating prolonged inflammation and delayed

healing. In contrast, *Satureja montana* L. hydrolate gel significantly reduced IL-6, TNF-α and CRP levels, suggesting effective inflammation modulation. These results align with the capacity of rosmarinic acid to modulate inflammatory responses, a primary compound in *Satureja montana* L.[21] Similarly, *Origanum vulgare* L. hydrolate gel reduced inflammatory markers, though less effectively than *Satureja montana* L.

Histological analysis revealed consistent healing patterns. The untreated group displayed dense inflammatory infiltration and delayed epithelialisation, mirroring persistently elevated inflammatory markers. Prolonged inflammation in untreated burns often hampers tissue regeneration and increases infection risks.[23] The *Satureja montana* L. group demonstrated advanced re-epithelialisation and reduced inflammatory infiltration by day 14, likely linked to cytokine modulation and oxidative stress reduction by rosmarinic acid.[24, 25] The *Origanum vulgare* L. group also showed improved epithelial restoration and reduced inflammatory cell presence, albeit to a lesser extent. These differences likely reflect variations in bioactive compound composition. While our histological evaluation using H&E staining provided valuable insights about morphological skin's conditions, additional markers specific to angiogenesis and tissue repair (eg VEGF, PDGF) were not assessed in this study.[26] Future research incorporating immunohistochemical analysis of these markers could offer a deeper understanding of the cellular mechanisms underlying the observed effects of hydrolates.

In summary, *Satureja montana* L. and *Origanum vulgare* L. hydrolate-based gels effectively modulate inflammation and promote tissue repair in burn wounds, with *Satureja montana* L. demonstrating superior outcomes. This efficacy may stem from its higher content of bioactive compounds, shaped by unique ecological conditions.[12] While the rabbit model offers valuable early-stage insights, its thinner epidermis, distinct collagen organisation and variations in inflammatory mediator expression limit direct extrapolation to human skin physiology. Consequently, *ex vivo* human skin studies and clinical trials would provide a more accurate representation of wound-healing mechanisms, as well as confirm the safety and efficacy of these hydrolate-based gels. Further investigation of the specific molecular pathways involved in inflammation control and tissue regeneration is warranted, clarifying how these treatments may best be applied in clinical practice. Building on these findings, hydrolate-based therapies present potential as promising alternatives to conventional burn treatments.

Conclusion

Study demonstrated that hydrolate-based gels derived from *Satureja montana* L. and *Origanum vulgare* L. effectively promoted burn wound healing. *Satureja montana* L. hydrolate, in particular, exhibited superior anti-inflammatory and regenerative properties, leading to enhanced epithelial regeneration and improved tissue integrity. These results suggest that hydrolates hold promise as complementary treatments for burn wound management. Future studies should aim to elucidate their underlying mechanisms and refine their use in clinical practice.

Ethics

The Bioethics Commission of the Institute of Translational Medicine and Biotechnology, Sechenov University approved this study (Protocol No 28), dated 21 October 2023. All procedures were conducted in strict compliance with the ILAR guidelines for the care and use of laboratory animals, the "International recommendations for conducting biomedical research using animals" (EEC, Strasbourg, 1985) and the "European convention for the protection of vertebrate animals for experimental and other scientific purposes" (EEC, Strasbourg, 1986).

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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