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Wound-healing potential of citrus lemon honey: Antioxidant, anti-inflammatory, antibacterial, and immunomodulatory effects on keratinocyte proliferation and migration

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Abstract

Honey from citrus limon is of interest for wound healing due to its antimicrobial, antioxidant, and immunomodulatory properties . This *in vitro* study investigates whether citrus lemon honey (CLH) can modulate key cellular and biochemical processes involved in wound healing, in comparison with Manuka honey. Antioxidant, anti-inflammatory, and antibacterial activities were evaluated alongside keratinocyte (HaCaT) proliferation and migration assays. Physicochemical analysis showed high water-soluble protein content (480.9 ± 28.4 mg equivalent [Eq] bovine serum albumin/100 g), acidic pH (3.4 ± 0.02), low moisture (19.5%), and moderate electrical conductivity (41.6 ± 2.61 µS/cm). In addition, CLH exhibited notable phenolic (313.6 ± 0.6 mg Eq gallic acid/100 g) and flavonoid contents (6.9 ± 2.6 mg Eq quercetin/100 g), accompanied by strong antioxidant activity (total antioxidant capacity: 3.5 ± 0.05 g Eq ascorbic acid/100 g). CLH demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria (minimum inhibitory concentrations of 0.8–3.1 w/w%), including clinically relevant wound-associated pathogens, highlighting its potential to reduce microbial burden and prevent infection in wound environments. Furthermore, CLH modulated nitric oxide production in lipopolysaccharide-activated THP-1 macrophages. Functionally, CLH promoted keratinocyte viability and migration in a concentration-dependent manner, with an approximately 20% increase in cell viability at 1–2 mg/mL and a 35% enhancement in migration, without cytotoxicity. These effects were comparable in trend, though less pronounced, to those observed with Manuka honey. Taken together, the combined antibacterial, anti-inflammatory, and pro-regenerative effects suggest that CLH may support wound healing by enhancing tissue repair and limiting infection. From a host–

microbe interaction perspective, the antibacterial and immunomodulatory effects of CLH suggest a coordinated role in wound healing, where reduced microbial burden and controlled regulation of inflammation may act synergistically to support tissue repair and restore skin integrity. However, further *in vivo* and clinical studies are required to confirm its therapeutic relevance.

Keywords: Citrus lemon honey; Immunomodulatory; Antibacterial; Antioxidant activity; Wound healing; HaCaT cells

1. Introduction

The skin is the largest organ of the body. It protects against environmental factors and plays essential roles in sensory perception, thermoregulation, immunological surveillance, fluid homeostasis, and self-healing.[1,2] Severe traumas, including tears, gashes, cuts, burns, abrasions, scrapes, and bruises, are often intensified by underlying conditions such as diabetes mellitus, stress, uremia, fibrosis, obesity, hypertension, poor nutrition, and immunocompromised states.[3]

Wound healing is a complex and dynamic process that involves hemostasis, inflammation, proliferation, and remodeling. Conventional treatments, such as antibiotics and synthetic dressings, face several limitations, including rising antimicrobial resistance, adverse side effects, and limited efficacy in promoting tissue regeneration.[4,5] These challenges emphasize the need to search for alternative or complementary wound care agents with proven bioactivity. Whole-plant extracts and other natural products are known to contain diverse therapeutic compounds, including antimicrobial, antioxidant, and anti-inflammatory agents, as well as stimulators of re-epithelialization and collagen formation. These bioactive constituents can act synergistically to enhance the wound healing process.[6–8] Among natural products, honey has long been appreciated for its role in maintaining health and treating various medical conditions.[9] It has been used for centuries in traditional medicine due to its wide range of therapeutic properties. Honey exhibits a broad spectrum of health-promoting effects, including immunomodulatory, antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities.[9] The botanical source and geographical origin strongly influence the chemical composition of honey and, hence, its efficacy. Generally, honey consists mainly of sugars, primarily D-glucose and D-fructose, along with small amounts of water and a variety of minor constituents such as amino acids, proteins, lipids, vitamins, minerals, and a complex mixture of phytochemicals, including polyphenols, flavonoids, terpenoids, and volatile compounds.[10]

Monofloral honeys, derived predominantly from the nectar of a single plant species, have attracted increasing scientific interest due to their distinctive chemical composition and therapeutic effects. Their clinical relevance is underlined by the approval of MEDIHONEY®, a medical-grade Manuka honey-based product, by the U.S. Food and Drug Administration in 2007 for the treatment of various types of wounds.[11] Within the wide range of botanical sources, citrus honeys, originating from the blossoms of *Citrus* species such as *Citrus limon,* have recently gained particular attention due to their unique phytochemical profile. Citrus honeys are rich in flavonoids, polyphenols, and vitamin C, bioactive constituents well known for their antioxidant and anti-inflammatory activities, which can reduce oxidative stress and support cellular repair mechanisms in damaged tissues.[1,12] Comparative studies of citrus honeys have demonstrated that lemon honey, in particular, exhibits high flavonoid content, strong protective effects against protein denaturation, and inhibitory activity against bacterial biofilm formation, suggesting potential mechanisms for reducing microbial burden and inflammation within wound environments.[1,2]

Despite the limited number of direct experimental evaluations of lemon honey in wound-healing models, the reported bioactivities provide a strong scientific rationale for its investigation as a natural adjunct in wound care. However, a clear research gap remains, as the mechanistic effects of citrus lemon honey (CLH) on key cellular processes involved in wound healing—particularly inflammation and re-epithelialization—have not been systematically investigated using well-defined *in vitro* models.

Therefore, we hypothesize that CLH can modulate inflammatory responses and enhance keratinocyte proliferation and migration, thereby influencing critical steps of the wound healing process, with effects comparable in trend to those of Manuka honey.

The present study aims to evaluate the physicochemical properties, antioxidant capacity, antibacterial activity, and wound healing potential of citrus honey. To address

them, HaCaT keratinocytes were selected as a well-established model for studying re-epithelialization through proliferation and migration assays, while THP-1-derived macrophages were used as a validated model to assess inflammatory responses, including nitric oxide (NO) production. These complementary *in vitro* systems provide a controlled framework to evaluate both proliferative and immunomodulatory aspects relevant to wound healing.

Our findings demonstrate that CLH exhibits potent antioxidant activity and significant antibacterial effects against both Gram-positive and Gram-negative bacteria. Furthermore, the honey modulated inflammatory responses in THP-1-derived macrophages. CLH enhanced key wound healing-related cellular responses by promoting keratinocyte viability and migration. CLH increased cell viability without cytotoxicity and significantly enhanced cell migration. These effects were comparable in trend, though less pronounced, to those observed with Manuka honey. These findings support the wound healing potential of CLH and validate its biological activity relative to the established Manuka honey standard.

2. Materials and methods

2.1. Honey sample source and storage

Citrus lemon honey used in this study was commercially obtained in 2023 from Honey Spring, a certified vendor based in Tulkarem, Northern West Bank. Manuka honey (Comvita®, New Zealand), a certified and internationally recognized medical-grade product, was used as a reference comparator. Both honey samples were procured commercially and handled under identical conditions as described in the following sections. Upon acquisition, samples were sealed in sterile, airtight metal containers and stored under dry, ambient conditions, protected from light and moisture, until further analysis. All handling procedures were performed to preserve physicochemical stability and minimize degradation prior to experimental use.

2.2. Antioxidant activity via 2,2-diphenyl-1-picrylhydrazyl

A modified version of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) microdilution assay was used to assess the free radical-scavenging capacity of the honey, as previously described.[6,13]

2.3. Total antioxidant capacity

Total antioxidant capacity (TAC) was evaluated using the phosphomolybdenum method according to the procedure described by Prieto *et al.*[14] Results were expressed as mg ascorbic acid (AA) equivalents per gram of honey, and all assays were performed in triplicate.

2.4. Quantification of total phenolics and flavonoids

Total phenolic content (TPC) and total flavonoid content (TFC) were determined using colorimetric assays, in accordance with established protocols.[15]

2.5. Evaluation of antibacterial properties

Streptococcus pneumoniae (ATCC 49619), *Klebsiella quasipneumoniae* (ATCC 700603), *Haemophilus influenzae* (ATCC 49247), and *Bacillus subtilis* (ATCC 6633) were used to evaluate the antibacterial activity of the citrus honey. All strains were obtained from the American Type Culture Collection, USA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using standard broth microdilution assays in 96-well plates.[13]

2.6. Culturing of human cell lines

Cells from the HaCaT human keratinocyte cell line (Cat. No. 300493, CLS Cell Lines Service GmbH, Germany) and the human THP-1 monocytic cell line (ATCC TIB-202) were used to examine the wound healing ability of the citrus honey. Both cell lines were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide using Dulbecco's modified Eagle medium (DMEM; Biological Industries, Israel) supplemented with 10% fetal calf serum, 1% non-essential amino acids, 1% glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin.

2.7. Assessment of cytotoxic and proliferative effects

The cytotoxic effects of CLH were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 20,000 HaCaT cells per well were seeded in 96-well plates and allowed to adhere for 24 h. Cells were then treated with CLH at concentrations ranging from 0 to 4,000 µg/mL for an additional 24 h, after which cell viability was evaluated using the MTT assay. For cell proliferation analysis, 5,000 cells per well were seeded and treated similarly but incubated for 72 h prior to MTT assessment, following the protocol described by Abu-Farich *et al.*[13]

2.8. *In vitro* wound healing assay (scratch assay)

HaCaT cells were seeded at a density of 200,000 cells per well in 24-well plates and incubated for 24 h until confluence was achieved. A linear scratch was then created using a sterile 200 µL pipette tip. Wells were gently washed with serum-free DMEM to remove detached cells. Triplicate wells were subsequently treated with 1, 2, or 4 mg/mL honey solutions or control medium. The percentage of wound closure was

calculated by comparing the wound area at 0, 24, and 48 h relative to the initial wound area at 0 h.

2.9. Nitric oxide inhibition

THP-1-derived macrophages were activated with lipopolysaccharide (LPS) and treated with CLH samples. Nitrite accumulation in the culture medium was quantified using the Griess assay, as previously described.[16]

2.10. Statistical analysis

All experimental data are presented as means ± standard deviation of three independent experiments. Group comparisons were made using unpaired Student's *t*-tests, with statistical significance set at $p < 0.05$.

3. Results and discussion

The wound-healing process involves multiple interdependent pathophysiological and immunological mediators that coordinate repair responses to various causes, such as trauma, surgery, and burns, ultimately restoring tissue integrity and barrier function.[17] A variety of medicinal approaches have been used to treat wounds. Anti-inflammatory and antimicrobial therapies are the most common treatments, as most wounds are susceptible to microbial infection and are often difficult to manage.[18] However, the rise of multidrug-resistant bacteria has made many conventional antimicrobial agents less effective in wound treatment. Thus, alternative therapeutic strategies have been explored. Honey, with its long history of use, has attracted increasing scientific attention over the past two to three decades.[18] It has been used for millennia in major traditional medical systems, including Greco-Arab and Islamic traditional medicine, traditional Chinese medicine, and Ayurveda for the treatment of wounds.[9,19] Citrus honey has been traditionally employed in skin and wound-related therapies. Our experimental findings support these traditional applications by demonstrating that CLH exhibits multiple wound-healing activities, including antioxidant, anti-inflammatory, proliferative, and antibacterial effects. Notably, the antibacterial activity is particularly relevant in the context of wound care, where microbial colonization and infection—especially by opportunistic and resistant pathogens—represent major barriers to effective healing. Importantly, wound healing is strongly influenced by dynamic host–microbe interactions, where microbial colonization, immune activation, and tissue repair processes are tightly interconnected. In this context, the dual antibacterial and anti-inflammatory properties of CLH may help restore balance in the wound microenvironment. By limiting microbial load while simultaneously modulating the host inflammatory response, CLH may help prevent the persistence of a pro-inflammatory state that delays healing.

3.1. Physicochemical properties of citrus honey

Table 1 summarizes the physicochemical characteristics of the citrus honey. High protein levels were measured, with a water-soluble protein content of 480.9 ± 28.4 mg bovine serum albumin equivalents per 100 g of honey. The total sugar content was 19.76 ± 0.3 g/100 g. The pH of the sample was 3.4 ± 0.02, consistent with honey's acidic nature and its contribution to antimicrobial activity. The Brix index was 79.9 ± 0.7, corresponding to the total soluble solids and indicating a high sugar concentration. The moisture content was relatively low (19.5%), falling within the acceptable range for unfermented honey and supporting its long-term stability. The electrical conductivity was 41.6 ± 2.61 µS/cm, classifying the sample within the typical range for nectar-derived honeys. Finally, the honey was visually characterized as extremely light-colored (white), suggesting a low pigment content due to its botanical origin. This pale coloration may also reflect a reduced concentration of carotenoids and other pigment-associated phytochemicals, which in turn could influence the antioxidant profile and bioactivity of the honey.[20,21]

The physicochemical properties of the CLH sample fall within the ranges established by the Codex Alimentarius and other international standards[22,23], as well as with other honey types.[7,8] Its low moisture, acidic pH, and moderate conductivity are characteristic of high-quality nectar honeys. The elevated protein content may indicate enzymatic richness, which can contribute to antioxidant and antimicrobial activity, hence, to its therapeutic value.

3.2. Antioxidant properties of citrus honey

Table 2 presents the TPC, TFC, TAC, and DPPH radical scavenging activity used to characterize the antioxidant properties of CLH. The TPC was 313.6 ± 0.6 mg gallic acid equivalents (GAE) per 100 g of honey, indicating a high level of polyphenolic compounds. The TFC was measured at 6.9 ± 2.6 mg quercetin equivalents per 100 g, reflecting a moderate flavonoid content. The TAC, assessed by the phosphomolybdenum assay, was 3.5 ± 0.05 g ascorbic acid equivalents per 100 g, indicating measurable antioxidant capacity.

The DPPH assay yielded a half-maximal inhibitory concentration (IC_{50}) of 10.6 ± 0.3 mg/mL, indicating moderate free radical scavenging activity. Lower IC_{50} values indicate higher antioxidant activity, and this result suggests that CLH exhibits moderate antioxidant activity, consistent with its phenolic and flavonoid composition.[24]

When compared to Manuka honey, which typically exhibits higher TPC (400–700 mg GAE/100 g) and stronger

Table 1. Physicochemical characteristics of citrus lemon honey

Water-soluble proteins	Total sugars	pH	Brix index	Moisture (%)	Electrical conductivity (µS/cm)	Color
480.9 ± 28.4	19.76 ± 0.3	3.4 ± 0.02	79.9 ± 0.7	19.5	41.6 ± 2.61	White

Notes: Water-soluble protein: mg equivalent bovine serum albumin/100 g; Total sugar: g equivalent glucose/100 g.

Table 2. Antioxidant activity and phytochemical composition of citrus honey

Parameter	DPPH IC_{50}	TFC	TPC	TAC
Value	10.6 ± 0.3	6.9 ± 2.6	313.6 ± 0.6	3.5 ± 0.05

Notes: DPPH IC_{50}: mg/mL; TFC: mg equivalent quercetin/100 g of honey; TPC: mg equivalent gallic acid/100 g of honey; TAC: g equivalent ascorbic acid/100 g of honey.
Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: Half-maximal inhibitory concentration; TAC: Total antioxidant capacity; TFC: Total flavonoid content; TPC: Total phenolic content.

radical scavenging activity (IC_{50} values often below 5 mg/mL)[25], the antioxidant potential of CLH was moderate but remains substantial. Notably, CLH values were comparable to those reported for other honeys, such as thyme and wildflower varieties, which generally fall within the range of 250–350 mg GAE/100 g and IC_{50} values between 9–12 mg/mL.[15,26] These comparisons highlight that while CLH may not reach the potency of Manuka honey, it demonstrates a consistent antioxidant profile relative to regional honeys, supporting its relevance as a locally available therapeutic agent.

Although the phenolic and flavonoid content likely contributes to the observed antioxidant activity, no formal correlation analysis between TPC and IC_{50} was performed, as the study aimed primarily at comparative characterization rather than mechanistic quantification.

3.3. Proliferative and cytotoxic effects of citrus lemon honey on HaCaT cells

The cytotoxic and proliferative effects of CLH were evaluated in comparison with Manuka honey across a range of concentrations (0–4 mg/mL) (Figures 1 and 2). As shown in Figure 1, neither honey showed significant cytotoxicity after 24 h. The cell proliferation assay showed that at baseline (0 mg/mL), cell viability was comparable among all groups and normalized to 100%. For prolonged treatment (Figure 2), treatment with CLH at 1 mg/mL significantly enhanced HaCaT cell viability to approximately 120% of control ($p < 0.05$), while Manuka honey induced a slightly higher proliferative response, reaching about 130% ($p < 0.05$). At 2 mg/mL, CLH maintained a moderate but significant proliferative effect (~115% of control), whereas Manuka honey exhibited a marked increase in cell viability, peaking at approximately 160% ($p < 0.05$), indicating a strong concentration-dependent stimulatory effect. In contrast, exposure to the highest tested concentration (4 mg/mL) reduced cell viability in both honeys, with CLH decreasing to ~80% and Manuka honey returning to near-control levels (~100%), suggesting potential cytotoxic or inhibitory effects at elevated concentrations. Overall, CLH demonstrated a clear pro-proliferative effect at low to intermediate concentrations, although its magnitude was consistently lower than that observed for Manuka honey. The significant enhancement of proliferation at low concentrations suggests a potential regenerative role. Keratinocyte proliferation and migration are vital for re-epithelialization, the phase of wound healing during which the epidermis is restored.[27,28] Hence, agents that can stimulate keratinocyte growth without inducing cytotoxicity are of great therapeutic interest.

Keratinocyte proliferation and migration are essential processes in re-epithelialization, the wound-healing phase that promotes wound closure and restores the epidermal barrier.[29] Numerous *in vitro* studies have shown that honey enhances keratinocyte proliferation and accelerates cell migration, with these effects being more strongly correlated with the phenolic and flavonoid content of honey than with its sugar composition alone.[30,31] Citrus honeys, including lemon honey, are particularly rich in polyphenols with potent antioxidant activity, which can decrease oxidative stress and modulate cellular signaling pathways involved in keratinocyte proliferation and migration.[31,32] In the

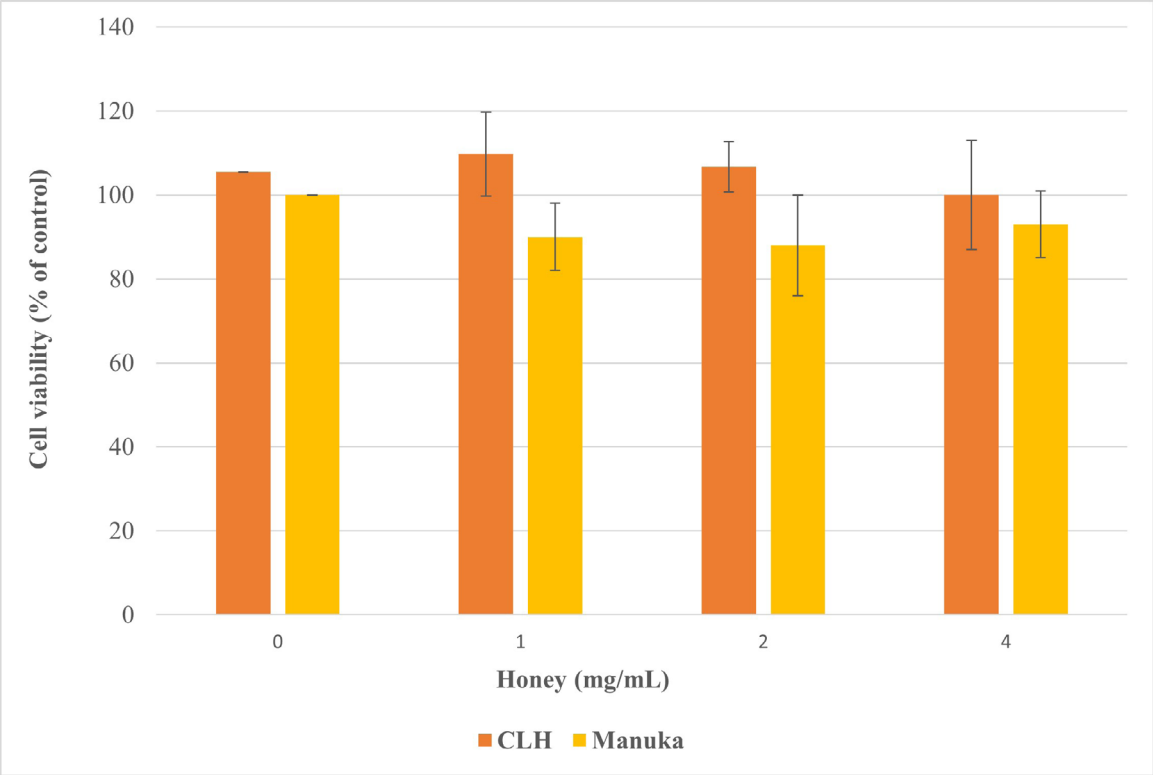


Figure 1. Cytotoxic effects of citrus lemon honey (CLH) on HaCaT cells. HaCaT cells were treated with CLH or Manuka honey at concentrations of 0, 1, 2, and 4 mg/mL for 24 h. Data are presented as a percentage of the control, with the mean ± standard deviation from three independent experiments.

present study, CLH significantly enhanced keratinocyte proliferative activity. These findings are consistent with our previous investigations on other monofloral honeys, including Medicago sativa and avocado honeys, which have demonstrated marked wound-healing efficacy through integrated antioxidant, anti-inflammatory, antibacterial, and proliferative mechanisms.[7,8] Across these studies, enhanced keratinocyte activity has been shown to be closely associated with rich phytochemical profiles, particularly phenolic and flavonoid compounds, suggesting that bioactive constituents common to honeys collectively contribute to improved re-epithelialization and wound closure.[7,8]

3.4. Effect of citrus lemon honey on HaCaT cell migration

Keratinocyte migration is a fundamental process during the re-epithelialization phase of wound healing. It enables epidermal repair following injury.[33] In the present study, untreated HaCaT keratinocytes were normalized to 100% migration, and the effects of CLH were evaluated in comparison with Manuka honey (positive control). Both honeys enhanced HaCaT cell migration in a concentration-dependent manner, as shown in Figure 3, with peak effects seen at low concentrations (1–2 mg/mL). CLH induced a moderate but consistent increase in cell migration compared to untreated controls. Manuka honey produced significantly higher migratory effects at the same concentrations, reaching nearly a two-fold increase relative to baseline, confirming its established pro-regenerative activity. The greater effect of Manuka honey on keratinocyte migration is consistent with previous *in vitro* studies demonstrating that Manuka honey accelerates wound closure and enhances keratinocyte motility by modulating inflammatory mediators, oxidative stress, and growth factor signaling.[34,35] Ranzato *et al.*[36] demonstrated that honey stimulates keratinocyte migration and induces cytoskeletal reorganization, promoting re-epithelialization independently of cell proliferation. These effects are partly attributed to honey-derived phenolic compounds, which regulate reactive oxygen species at non-toxic levels conducive to cell signaling.[9,36]

Citrus honey, while less potent than Manuka honey, still significantly promoted migration at 2 mg/mL. This effect is consistent with the presence of flavonoids and phenolic compounds in citrus honey, which are known to influence oxidative and inflammatory pathways involved in keratinocyte migration.[9,37,38] Previous *in vitro* and *in vivo*

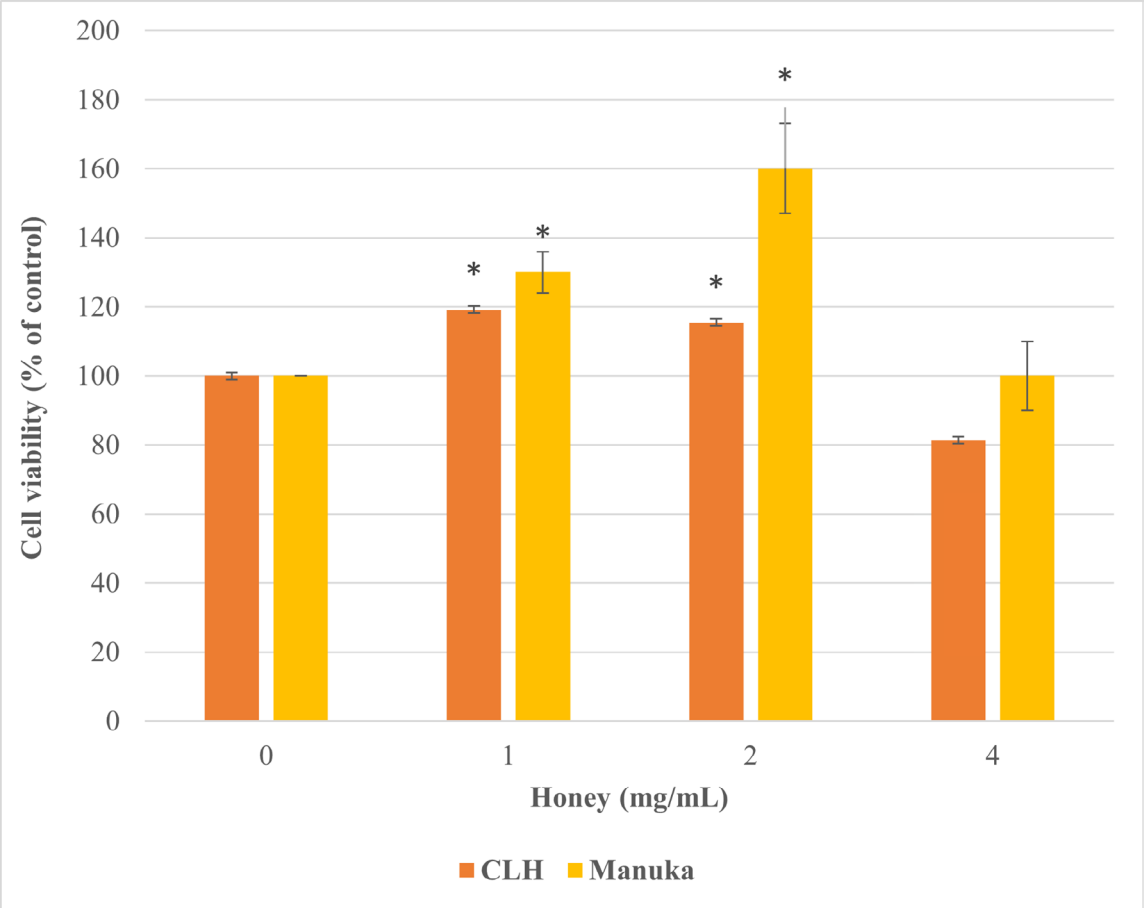


Figure 2. Cell proliferation effects of citrus lemon honey (CLH) on HaCaT cells. HaCaT cells were treated with CLH at concentrations of 0, 1, 2, and 4 mg/mL for 72 h. Data are presented as a percentage of the control, with the mean ± standard deviation from three independent experiments.
Note: *$p < 0.05$ vs. the respective control (0 mg/mL).

studies have reported reduced levels of pro-inflammatory cytokines following honey treatment.[9,38] At 4 mg/mL, a reduction in migratory activity was observed for both honeys. A similar biphasic response has been reported previously, where higher honey concentrations impaired cell migration, likely due to osmotic or metabolic stress.[35,39]

These results suggest that CLH has significant effects on proliferative and migratory behavior in HaCaT keratinocytes. The findings support the view that honey-based therapies promote re-epithelialization by enhancing keratinocyte proliferation and migration.[33] Further mechanistic studies are warranted to elucidate the signaling pathways involved and to validate these findings in *in vivo* wound models.

3.5. Inhibition of nitric oxide production in lipopolysaccharide-stimulated macrophages

The anti-inflammatory activity of CLH was assessed by measuring NO production in LPS-stimulated THP-1-derived macrophages. As shown in Figure 4, treatment with LPS increased NO levels significantly to 440 ± 40% relative to the untreated control (100%). Treatment with 1–2 mg CLH/mL markedly reduced NO production to 110 ± 15% of control levels. This suppression of NO is strongly correlated with the honey's TPC of 47.37 mg/g. Polyphenols are known to interfere with key inflammatory pathways by downregulating inducible NO synthase (iNOS) expression and inhibiting the nuclear factor (NF)-κB signaling cascade, which is essential for pro-inflammatory gene transcription.[9,40,41] Ellagic acid, for example, has been shown to inhibit NO release and pro-inflammatory cytokine production in LPS-stimulated macrophages by targeting the NF-κB and mitogen-activated protein kinase pathways.[42,43] Similarly, caffeic acid and pinocembrin have been documented to reduce iNOS expression and suppress oxidative burst in immune cells, mitigating the inflammatory response.[44] Flavonols such as kaempferol and quercetin also exhibit potent antioxidant

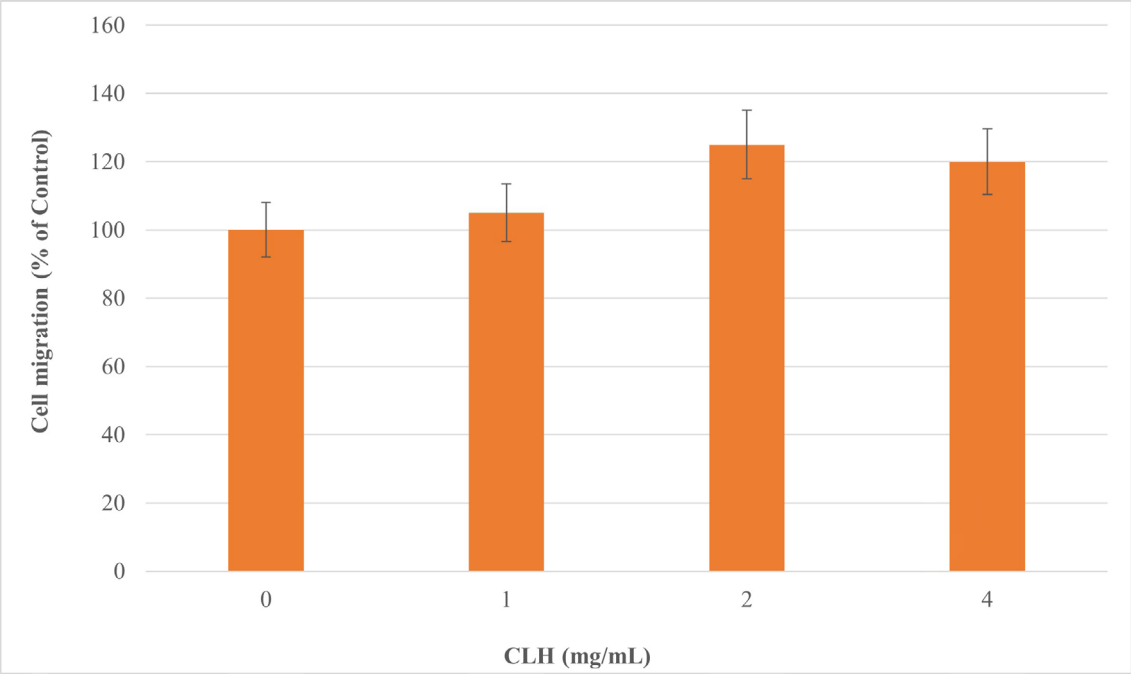


Figure 3. Citrus lemon honey (CLH) promotes HaCaT cell migration. Wound closure (%) was measured at 48 h after treatment with 1, 2, 4 mg/mL honey, normalized to untreated controls (100%). Data are shown as mean ± standard deviation of three independent experiments.
Note: *$p < 0.05$ vs. the respective control (0 mg/mL).

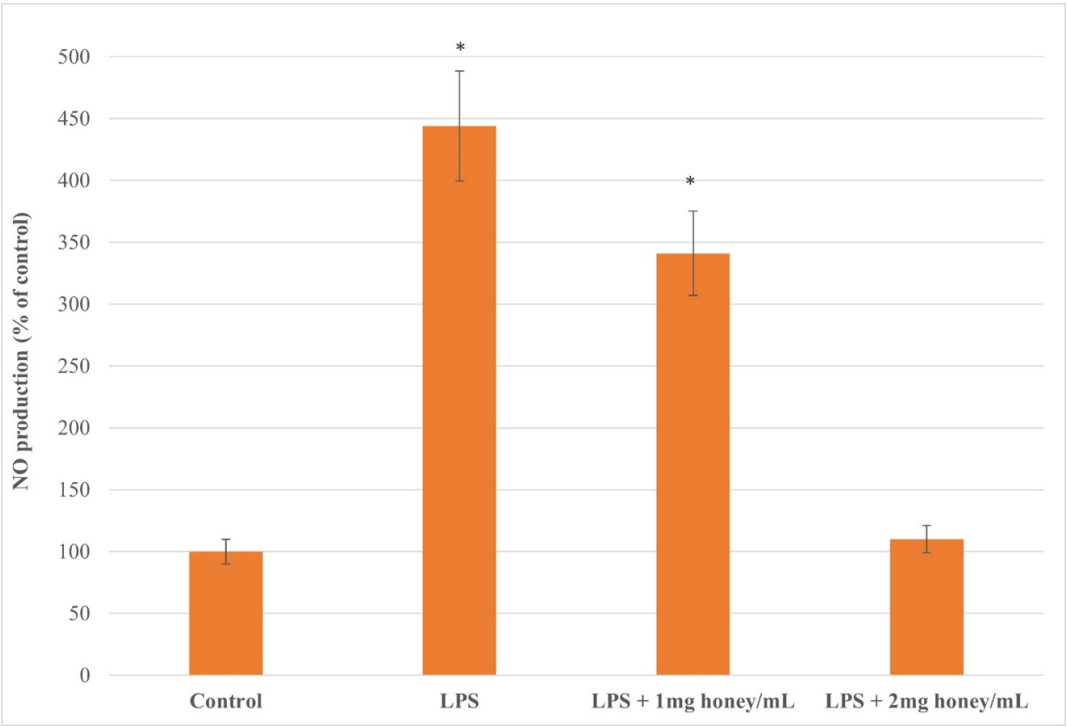


Figure 4. Anti-inflammatory effect of citrus lemon honey (CLH) in lipopolysaccharide (LPS)-stimulated macrophages. CLH attenuates LPS-induced inflammation in THP-1-derived macrophages. Cells were treated with LPS, with or without 1 or 2 mg/mL CLH for 72 h. Inflammatory response was quantified and expressed as a percentage relative to the untreated control (set at 100%). Data represent the mean ± standard deviation of three independent experiments.
Note: *$p < 0.05$ vs. control. Abbreviation: NO: Nitric oxide.

activity, scavenging reactive oxygen species and indirectly inhibiting NO synthesis.[45]

Nitric oxide is a central mediator of the innate immune response, produced by activated macrophages during the inflammatory phase of wound healing. While NO plays an essential role in host defense through antimicrobial activity and signaling, its excessive production can lead to prolonged inflammation, oxidative damage, and impaired tissue repair.

In this context, CLH's ability to markedly reduce NO production suggests a modulatory, rather than purely inhibitory, effect on macrophage activation. This controlled regulation may help maintain the balance between effective immune defense and inflammation resolution, which is critical for proper progression from the inflammatory to the proliferative phase of wound healing.[44,45]

The observed inhibition of NO production in LPS-stimulated macrophages can be attributed to the high polyphenolic content of citrus honey. These findings support its potential role as a natural therapeutic agent for modulating macrophage-mediated inflammation and promoting wound healing, consistent with previous reports on honey-derived polyphenols.[40,41]

3.6. Antibacterial activity of citrus lemon honey

The antibacterial properties of CLH were assessed against a panel of Gram-positive and Gram-negative bacterial strains using MIC and MBC assays (Figure 5). Among Gram-positive bacteria, Streptococcus pneumoniae showed the highest sensitivity, with both MIC and MBC values of 1.56% (w/w). Bacillus subtilis displayed moderate sensitivity, with MIC and MBC values of 1.6% (w/w) and 3.2% (w/w), respectively. For Gram-negative bacteria, Klebsiella quasipneumoniae was the most susceptible, with both MIC and MBC values of 0.8% (w/w). Haemophilus influenzae was comparatively less sensitive, showing MIC and MBC values of 3.1% (w/w) and 6.2% (w/w), respectively.

These findings confirm that CLH exhibits broad-spectrum antibacterial activity, in agreement with earlier studies demonstrating honey's effectiveness against both Gram-positive and Gram-negative pathogens, including multidrug-resistant strains such as Staphylococcus aureus and Pseudomonas aeruginosa. Importantly, several of these microorganisms are commonly associated with wound infections or secondary colonization, suggesting that CLH may play a role in controlling microbial load within the wound microenvironment.[7,8,13,14] The antimicrobial activity of honey is attributed to multiple factors. These include low pH, osmotic stress from high sugar content, hydrogen peroxide (H_2O_2) production, and the presence of phenolics and antimicrobial peptides. From a clinical perspective, controlling bacterial growth in wounds is essential to prevent biofilm formation, chronic inflammation, and delayed healing. The observed MIC values (0.8–3.1%) indicate that CLH exerts antibacterial activity at relatively low concentrations, supporting its potential as a topical antimicrobial agent for wound management. Furthermore, the demonstrated activity against both Gram-positive and Gram-negative bacteria suggests that CLH could provide broad-spectrum antimicrobial coverage, particularly valuable in polymicrobial wound infections.[46,47]

The effectiveness of honey against multidrug-resistant bacteria has been recorded in several studies.[7,8,48] Manuka honey, for instance, was found to restore bacterial susceptibility when combined with antibiotics, a tool that could help reduce antibiotic resistance.[49–51] This synergistic property further underscores honey's clinical potential in treating infected wounds. It is worth noting that the antimicrobial efficacy of honey varies depending on its botanical source and its active compounds. For instance, MEDIHONEY®, derived from Manuka honey, contains high levels of methylglyoxal (MGO), which is responsible for its non-peroxide antibacterial action. In contrast, Revamil honey relies on H_2O_2 production and bee defensin-1, demonstrating a peroxide-dependent mechanism.[52] Interestingly, MGO can degrade key enzymatic proteins like glucose oxidase and bee defensin-1, suggesting that both mechanisms may not coexist effectively in the same honey type.[53,54] In this context, the potent antibacterial activity observed for CLH, particularly against B. subtilis and S. pneumoniae, suggests the presence of active antimicrobial compounds and possibly peroxide-dependent pathways, as supported by the observed sensitivity of Gram-positive strains. Further phytochemical characterization is needed to elucidate the exact compounds responsible for this activity and their stability under physiological conditions.

Overall, these findings reinforce the microbiological relevance of CLH, highlighting its dual role in wound healing: promoting tissue repair while simultaneously limiting infection by wound-associated pathogens. The interplay between antimicrobial activity and immune regulation is a key determinant of wound healing outcomes. Persistent microbial presence can prolong inflammation and impair tissue repair, while excessive inflammation can further damage tissue and delay regeneration. In this context, the antibacterial activity of CLH may reduce pathogen-associated stimuli, while its anti-inflammatory and immunomodulatory effects may limit excessive host responses. This coordinated action highlights the potential of CLH to modulate host–microbe interactions, favoring

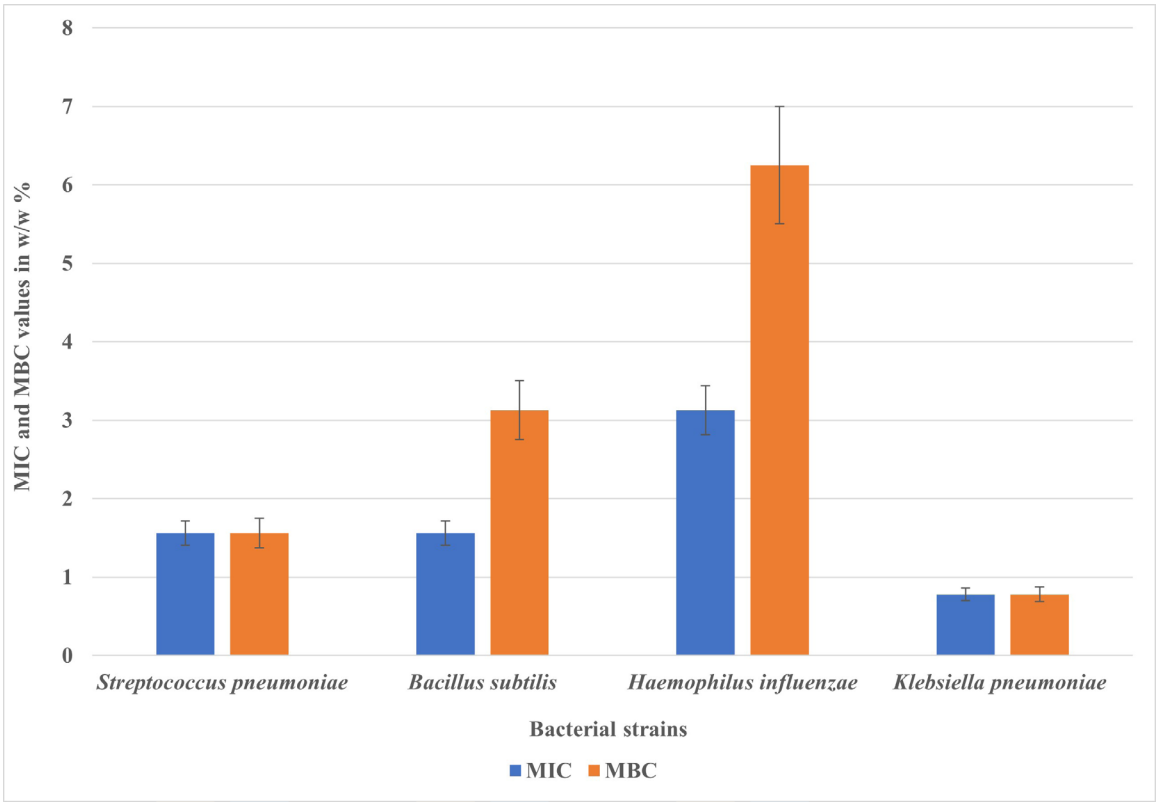


Figure 5. Antibacterial activity of citrus lemon honey (CLH). Antibacterial activity of CLH against various bacterial strains (minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] values in w/w%). The data presented are the mean of three independent experiments.

progression toward tissue repair rather than chronic inflammation.

4. Conclusion

Citrus lemon honey represents a rich natural source of bioactive compounds with antioxidant, anti-inflammatory, antibacterial, and wound-healing-related properties. The present study provides a comprehensive characterization of CLH and demonstrates its biological activity in *in vitro* models relevant to wound healing, rather than direct therapeutic efficacy. Physicochemical analysis showed low moisture content, an acidic pH, and elevated protein levels, which collectively create an environment that supports the stability and bioactivity of its active compounds.

Biological assays demonstrated that CLH exerts potent antioxidant effects, reduces NO production in LPS-stimulated macrophages, and promotes keratinocyte proliferation and migration at appropriate concentrations. Importantly, the observed broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria underscores its potential to control wound-associated pathogens. This antimicrobial effect is particularly relevant for preventing infection, reducing microbial burden, and limiting complications such as delayed healing and chronic wound formation. These findings suggest, but do not confirm, that honey's polyphenolic compounds may contribute to cellular protection against oxidative stress and to the modulation of inflammatory pathways, thereby facilitating tissue repair. In addition, broad-spectrum antibacterial activity was observed against both Gram-positive and Gram-negative strains, with the data suggesting that multiple factors, including acidity, osmotic effects, enzymatic hydrogen peroxide release, and phenolic compounds, contribute to its activity. Although these interactions are promising, further mechanistic studies are required to confirm the exact pathways involved. The combined antimicrobial and regenerative properties suggest that CLH may serve as a multifunctional wound-care agent, addressing both infection control and tissue repair—two critical and interdependent aspects of wound healing. The observed reduction in NO levels further supports an immunomodulatory role, in which CLH may regulate innate immune responses by controlling macrophage-driven inflammation. This is particularly important in

wound healing, where a balanced inflammatory response is required to prevent chronic inflammation while still enabling effective antimicrobial defense.

Importantly, the present study is limited by its reliance on *in vitro* models, which do not fully recapitulate the complexity of wound healing *in vivo*, including tissue architecture, immune system interactions, and systemic factors. Therefore, the observed effects should be interpreted with caution when extrapolating to clinical settings.

Based on our results, the findings support the biological activity of CLH *in vitro* but do not constitute validation of its traditional use or clinical efficacy in wound healing and should be considered as preliminary evidence requiring further investigation. Nevertheless, *in vitro* results represent only an initial step, and translation to clinical application will require *in vivo* animal models and controlled clinical trials. Future studies should also investigate the molecular targets of key polyphenols, the dose-dependent effects on different skin cell populations, and potential synergistic interactions with conventional wound-care treatments. Further phytochemical characterization using advanced chromatographic and spectroscopic techniques is required to identify the specific compounds responsible for the observed biological activities.

Overall, the present findings suggest that CLH holds promise as a natural adjunct for topical wound care, owing to its combined antimicrobial, immunomodulatory, and pro-regenerative properties. While these *in vitro* results are encouraging, clinical translation requires further validation in animal models and human studies to establish efficacy, safety, and optimal application strategies.

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Conflict of interest

Bashar Saad is the Editorial Board Member of this journal and the Guest Editor of this special issue but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Availability of data

Data are available upon reasonable request to the corresponding author.

References

1. Chylińska N, Maciejczyk M. Hyaluronic Acid and Skin: Its Role in Aging and Wound-Healing Processes. *Gels*. 2025;11(4):281.

   doi: 10.3390/gels11040281

2. Baker P, Huang C, Radi R, Moll SB, Jules E, Arbiser JL. Skin Barrier Function: The Interplay of Physical, Chemical, and Immunologic Properties. *Cells*. 2023;12(23):2745.

   doi: 10.3390/cells12232745

3. Summer M, Ali S, Fiaz U, Hussain T, Khan RR, Fiaz H. Revealing the molecular mechanisms in wound healing and the effects of different physiological factors including diabetes, age, and stress. *J Mol Histol*. 2024;55(5):637-654.

   doi: 10.1007/s10735-024-10223-3

4. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. *Adv Ther*. 2017;34(3):599–610.

   doi: 10.1007/s12325-017-0478-y

5. Blackburn J, Ousey K, Rippon M, Cutting K, White R. Applying antimicrobial strategies in wound care practice: a review of the evidence. *Int Wound J*. 2025;22(6):e70684.

   doi: 10.1111/iwj.70684

6. El-yagoubi K, Masalha M, Abu-Farich B, Touzani S, Lyoussi B, Saad B. Antibacterial Activity, Antioxidant Benefits, Chemical Composition, and Physicochemical Properties of Ziziphus jujuba Honey from Five Distinct Regions in Morocco. *J Pure Appl Microbiol*. 2025;19(2):1358-1370.

   doi: 10.22207/JPAM.19.2.39

7. Jarar D, Kmail A, Masalha M, *et al.* Wound Healing

Mechanisms of Medicago sativa Honey: Antioxidant, Anti-inflammatory, Antibacterial and Phytochemical Characterisation. *J Nat Remedies*. 2025:2381-2395.

doi: 10.18311/jnr/2025/50943

8. Jarar D, Imtara H, Kmail A, *et al.* Experimental Evaluation of Avocado Honey's Wound Healing Properties: Physicochemical, Antibacterial, Anti-inflammatory, and Proliferative Effects Combined with *In Silico* Toxicity and Molecular Docking Analysis. *J Ethnopharmacol.* 2025;358:121038.

   doi: 10.1016/j.jep.2025.121038

9. Saad, B. Immunomodulatory and Anti-Inflammatory Properties of Honey and Bee Products. *Immuno*. 2025;5(2):19.

   doi: 10.3390/immuno5020019

10. Wang H, Li L, Lin X, Bai W, Xiao G, Liu G. Composition, functional properties and safety of honey: a review. *J Sci Food Agric.* 2023;103(14):6767–6779.

    doi: 10.1002/jsfa.12720

11. Mărgăoan R, Topal E, Balkanska R, *et al.* Monofloral honeys as a potential source of natural antioxidants, minerals and medicine. *Antioxidants*. 2021;10(7):1023.

    doi: 10.3390/antiox10071023

12. Fratianni F, Amato G, d'Acierno A, Panfili G, Coppola R, Nazzaro F. *In vitro* prospective healthy and nutritional benefits of different Citrus monofloral honeys. *Sci Rep*. 2023;13(1):1088.

    doi: 10.1038/s41598-023-27802-1

13. Abu-Farich B, Masalha M, Hamarshi H, *et al. In vitro* evaluation of Hyphaene thebaica honey as a multitarget therapeutic product. *Microbes Immun*. 2025;2(1):78–91.

    doi: 10.36922/mi.4994

14. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269(2):337-341.

    doi: 10.1006/abio.1999.4019

15. Abu-Farich B, Masalha M, Egbaria E, *et al.* Physicochemical properties, chemical composition, antioxidant properties, and antibacterial effects of four Palestinian honey varieties. *J Pure Appl Microbiol*. 2024;18(4):2315-2327.

    doi: 10.22207/JPAM.18.4.03

16. Vargas-Maya NI, Padilla-Vaca F, Romero-González OE, *et al.* Refinement of the Griess method for measuring nitrite in biological samples. *J Microbiol Methods.* 2021;187:106260.

    doi: 10.1016/j.mimet.2021.106260

17. Peña OA, Martin P. Cellular and molecular mechanisms of skin wound healing. *Nat Rev Mol Cell Biol*. 2024;25(8):599-616.

    doi: 10.1038/s41580-024-00715-1

18. Tashkandi H. Honey in wound healing: An updated review. *Open Life Sci*. 2021;16(1):1091-1100.

    doi: 10.1515/biol-2021-0084

19. Hossain Md. Lokman L, Lim YL, Katherine H, Hettiarachchi D, Locher C. Honey-Based Medicinal Formulations: A Critical Review. *Appl Sci.* 2021;11(11):5159.

    doi: 10.3390/app11115159

20. Sawarkar, A.B. Color intensity and antioxidant properties of honey produced from North Maharashtra, India. *J Food Sci*. 2025;62(12):2312-2320.

    doi: 10.1007/s13197-024-06170-2

21. Pontis, J.A, Costa, L.A, Silva, S.J, Flach, A. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Sci Technol.* 2014;34(1):69-73.

    doi: 10.1590/S0101-20612014005000015

22. European Commission. Council Directive 2001/110/EC of December 20, 2001, relating to honey. *Off J Eur Communities.* 2002;L10:47-52.

23. Bogdanov S, Ruoff K, Oddo LP. Physico-chemical methods for the characterisation of unifloral honeys: a review. *Apidologie*. 2004;35(Suppl. 1):S4-17.

    doi: 10.1051/apido:2004047

24. Martinez-Morales F, Alonso-Castro A., Zapata-Morales JR, Carranza-Álvarez C, Aragon-Martinez OH. Use of standardized units for a correct interpretation of IC50 values obtained from the inhibition of the DPPH radical by natural antioxidants. *Chem Pap*. 2020;74(10):3325-3334.

    doi: 10.1007/s11696-020-01161-x

25. Tananaki C, Rodopoulou MA, Dimou M, Kanelis D, Liolios V. The total phenolic content and antioxidant activity of nine monofloral honey types. *Appl Sci*. 2024;14(10):4329.

    doi: 10.3390/app14104329

26. Imtara H, Elamine Y, Lyoussi B. Physicochemical characterization and antioxidant activity of Palestinian honey samples. *Food Sci Nutr*. 2018;6(8):2056-2065.

    doi: 10.1002/fsn3.754

27. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. *Physiol Rev*. 2018;99(1):665-706.

    doi: 10.1152/physrev.00067.2017

28. Smith J, Rai V. Novel Factors Regulating Proliferation, Migration, and Differentiation of Fibroblasts, Keratinocytes, and Vascular Smooth Muscle Cells during Wound Healing. *Biomedicines*. 2024;12(9):1939.

doi: 10.3390/biomedicines12091939

29. Pastar I, Stojadinovic O, Yin NC, *et al.* Epithelialization in wound healing: a comprehensive review. *Adv Wound Care*. 2014;3(7):445-64.

doi: 10.1089/wound.2013.0473

30. Almasaudi SB, Al-Nahari AA, El Sayed M, *et al.* Antimicrobial effect of different types of honey on Staphylococcus aureus. *Saudi J Biol Sci*. 2017;24(6):1255-61.

doi: 10.1016/j.sjbs.2016.08.007

31. Kassim M, Achoui M, Mustafa MR, Mohd MA, Yusoff KM. Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate *in vitro* anti-inflammatory activity. *Nutr Res*. 2010;30(9):650-659.

doi: 10.1016/j.nutres.2010.08.008

32. Alvarez-Suarez JM, Giampieri F, Battino M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. *Curr Med Chem*. 2013;20(5):621-638.

doi: 10.2174/092986713804999358

33. Gushiken LF, Beserra FP, Bastos JK, Jackson CJ, Pellizzon CH. Cutaneous wound healing: An update from physiopathology to current therapies. *Life*. 2021;11(7):665.

doi: 10.3390/life11070665

34. Ebadi P, Fazeli M. Evaluation of the potential *in vitro* effects of propolis and honey on wound healing in human dermal fibroblast cells. *S Afr J Bot*. 2021;137:414-422.

doi: 10.1016/j.sajb.2020.10.003

35. Scepankova H, Combarros-Fuertes P, Fresno JM, *et al.* Role of honey in advanced wound care. *Molecules*. 2021;26(16):4784.

doi: 10.3390/molecules26164784

36. Ranzato E, Martinotti S, Burlando B. Epithelial mesenchymal transition traits in honey-driven keratinocyte wound healing: Comparison among different honeys. *Wound Repair Regen*. 2012;20(5):778-785.

doi: 10.1111/j.1524-475X.2012.00825.x

37. Tlak Gajger I, Dar SA, Ahmed MM, Aly MM, Vlainić J. Antioxidant capacity and therapeutic applications of honey: Health benefits, antimicrobial activity and food processing roles. *Antioxidants.* 2025;14(8):959.

doi: 10.3390/antiox14080959

38. Majtan J. Honey: an immunomodulator in wound healing. *Wound Repair Regen*. 2014;22(2):187-192.

doi: 10.1111/wrr.12117

39. Jull AB, Cullum N, Dumville JC, Westby MJ, Deshpande S, Walker N. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev*. 2015;3:CD005083.

doi: 10.1002/14651858.CD005083.pub4

40. Ling Y, Feng Y, Li Z. Cheng L, Zhang X, Ren D. Anti-Inflammatory Mechanism of Walnut Meal Peptide/ Ellagic Acid Nanoparticles on LPS-Stimulated RAW264.7 Cells by Inhibition of NF-κB and MAPK Activation. *Int J Pept Res Ther*. 2023;29(99).

doi: 10.1007/s10989-023-10571-w

41. Hu WH, Dai DK, Zheng BZ, *et al.* The binding of kaempferol-3-O-rutinoside to vascular endothelial growth factor potentiates anti-inflammatory efficiencies in lipopolysaccharide-treated mouse macrophage RAW264. 7 cells. *Phytomedicine*. 2021;80:153400.

doi: 10.1016/j.phymed.2020.153400

42. Ramos-Torrecillas J, González-Acedo A, Melguizo-Rodríguez L, *et al.* Anti-Inflammatory and Antimicrobial Effect of Ellagic Acid and Punicalagin in Dermal Fibroblasts. *Int J Mol Sci*. 2025;26(17):8681.

doi: 10.3390/ijms26178681

43. Ardah MT, Eid N, Kitada T, Haque, M.E. Ellagic acid prevents α-synuclein aggregation and protects sh-sy5y cells from aggregated α-synuclein-induced toxicity via suppression of apoptosis and activation of autophagy. *Int J Mol Sci*. 2021;22(24):13398.

doi: 10.3390/ijms222413398

44. Alam M, Ahmed S, Elasbali AM, *et al.* Therapeutic implications of caffeic acid in cancer and neurological diseases. *Front Oncol.* 2022;12:860508.

doi: 10.3389/fonc.2022.860508

45. Speisky H, Arias-Santé MF, Fuentes J. Oxidation of quercetin and kaempferol markedly amplifies their antioxidant, cytoprotective, and anti-inflammatory properties. *Antioxidants*. 2023;12(1):155.

doi: 10.3390/antiox12010155

46. Wasihun AG, Kasa BG. Evaluation of antibacterial activity of honey against multidrug resistant bacteria in Ayder Referral and Teaching Hospital, Northern Ethiopia. *SpringerPlus*. 2016;5(1):842.

doi: 10.1186/s40064-016-2493-x

47. Almasaudi S. The antibacterial activities of honey. *Saudi J Biol Sci*. 2021;28(4):2188-96.

doi: 10.1016/j.sjbs.2020.10.017

48. Machado A, Toubarro D, Baptista J, Tejera E, Álvarez-Suárez JM. Selected honey as a multifaceted antimicrobial agent: review of compounds, mechanisms, and research challenges. *Future Microbiol*. 2025;20(7-9):589-610.

doi: 10.1080/17460913.2025.2498233

49. Sekar M, Zuraini NZ, Rani NN, Lum PT, Gan SH. Antimicrobial properties of honey. In: *Honey: Composition*

and Health Benefits. John Wiley & Sons, Ltd. 2023:186-196.

doi: 10.1002/9781119113324.ch13

50. Ogwu MC, Izah SC. Honey as a natural antimicrobial. *Antibiotics*. 2025;14(3):255.

doi: 10.3390/antibiotics14030255

51. Liu M, Lu J, Müller P, *et al.* Antibiotic-specific differences in the response of Staphylococcus aureus to treatment with antimicrobials combined with manuka honey. *Front Microbiol*. 2015;5:779.

doi: 10.3389/fmicb.2014.00779

52. Kwakman PH, Te Velde AA, de Boer L, Vandenbroucke-Grauls CM, Zaat SA. Two major medicinal honeys have different mechanisms of bactericidal activity. *PloS ONE*. 2011;6(3):e17709.

doi: 10.1371/journal.pone.0017709

53. Majtan J, Bohova J, Prochazka E, Klaudiny J. Methylglyoxal may affect hydrogen peroxide accumulation in manuka honey through the inhibition of glucose oxidase. *J Med Food*. 2014;17(2):290-293.

doi: 10.1089/jmf.2012.0201

54. Kaźmierczak-Barańska J, Karwowski BT. The Antioxidant Potential of Commercial Manuka Honey from New Zealand-Biochemical and Cellular Studies. *Curr Issues Mol Biol*. 2024;46(7):6366-6376.

doi: 10.3390/cimb46070380