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Comparative effects of pistachio hull hydroalcoholic extract and lemongrass essential oil on excisional skin wound healing, in pigeon (*Columba livia*)

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Abstract

Cutaneous wound healing is a complex biological process that requires a well-regulated inflammatory response. Herbal products with antioxidant and anti-inflammatory properties have recently attracted considerable attention as alternative topical wound-healing agents. The present study aimed to compare the effects of hydroalcoholic pistachio hull (*Pistacia vera*) extract and lemongrass (*Cymbopogon citratus*) essential oil on excisional skin wound healing in pigeons (*Columba livia*). Eighteen clinically healthy adult pigeons were randomly allocated into six groups, including untreated control, vehicle control, 5% and 10% pistachio hull extract ointments, and 5% and 10% lemongrass essential oil ointments. Standardized full-thickness skin wounds were created on the dorsal cervical region, and topical treatments were applied once daily for 14 days. The relative mRNA expression of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-10 was determined using quantitative real-time PCR. Molecular analysis demonstrated that pistachio hull extract and lemongrass essential oil significantly downregulated the pro-inflammatory cytokines IL-6 and TNF-α while upregulating the anti-inflammatory cytokine IL-10, indicating effective modulation of the inflammatory phase of wound repair. Overall, the findings suggest that topical application of hydroalcoholic pistachio hull extract and lemongrass essential oil, particularly at a concentration of 10%, enhances cutaneous wound healing in pigeons by regulating inflammatory cytokine expression. These natural products may represent promising topical therapeutic agents for wound management in avian medicine and warrant further investigation through histopathological and clinical studies.

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Introduction

The skin is the largest organ of the body and the first line of defence against microorganisms. Although bacteria have difficulty penetrating healthy skin, when damaged, the underlying layers are exposed to pathogens and can lead to bacterial invasion (Goli *et al*., 2005). Wound healing is the result of a series of processes that ultimately restore lost tissue as much as possible. Wound healing is a dynamic process involving three stages: blood clotting, inflammation, and proliferation or formation of scar tissue. Each stage of the wound healing process begins immediately after the previous stage is completed, and the length of time spent in each stage will vary depending on the type of wound, its location, and the tissue that has been damaged (Eming *et al*., 2007).

With the advancement of medical science, the adverse effects of chemical medication have become more apparent than ever, and in contrast, the use of herbal medicines and substances has received more attention day by day. The pistacia belongs to the Anacardiaceae family, which grows annually in the Mediterranean basin to central Asia, especially in Iran, Turkey, Iraq, and Saudi Arabia. This herb has some properties such as sedative, anti-inflammatory, antimicrobial, antifungal, and antihyperlipidemic features. It has also been reported that this plant has beneficial therapeutic effects in healing full-thickness skin wounds and treating burns (Farahpour *et al*., 2015). The green skin (hull) of pistachios contains a large amount of phenolic compounds, which is significant compared to other substances (Goli *et al*., 2005). In Iran, more than 400,000 tons of pistachio hulls are obtained yearly after peeling pistachios (Mokhtarpour *et al*., 2014). Due to the high production of pistachio hulls in Iran and the presence of antioxidant compounds in them, which have been shown in previous studies, it is possible to produce natural antioxidants from pistachio hulls industrially (Rajaei *et al*., 2010).

Lemongrass (*Cymbopogon citratus*), a member of the Poaceae family, is a widely recognized medicinal and aromatic plant cultivated across tropical and subtropical regions of Asia, South America, and Africa (Shah *et al*., 2011). The plant has been extensively utilized in traditional medicine owing to its abundance of bioactive secondary metabolites, which are considered responsible for its diverse pharmacological properties. Historically, lemongrass has been employed in the treatment of various ailments, including fever, cough, influenza, leprosy, malaria, and a range of gastrointestinal disorders. Although its ethnomedicinal applications are well documented, scientific evidence supporting its therapeutic efficacy remains relatively limited. Consequently, the lack of comprehensive experimental and clinical investigations has restricted the translation of its medicinal potential into evidence-based clinical applications (Aw, 1966).

So far, no research has been conducted to evaluate the effects of pistachio hull hydroalcoholic extract and lemongrass essential oil on excisional skin wound healing in pigeons. Therefore, the purpose of the present study was to investigate this issue.

Materials and Methods

This study was performed during the winter of 2024 at the Department of Clinical Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies. The experimental protocol was reviewed and approved by the Institutional Ethics Committee of the university under the approval code IR.AUSMT.REC.1403.10. All experimental procedures were conducted in accordance with the approved ethical guidelines, and the rights and welfare of the animals were strictly observed throughout the study.

The pigeons used in this experiment were procured from domestic bird breeding facilities in Amol, northern Iran. A total of 18 clinically healthy, unsexed adult pigeons, with a mean body weight of approximately 280 g, were selected following a thorough physical examination. The birds were housed in stainless-steel cages measuring 100 × 60 × 60 cm and were provided with a standard commercial pelleted diet and unrestricted access to fresh drinking water. Before initiation of the experimental procedures, all pigeons underwent a 7-day acclimatization period under these housing conditions.

Pigeons were randomly allocated into six groups, which included: an untreated control group (creation of an experimental wound without any intervention or treatment) and a vehicle control group (creation of an experimental wound with a base ointment without extract).

Also, treatment groups 3 to 6 (after creating experimental wounds) received daily ointments of 5% pistachio extract, 10% pistachio extract, 5% lemongrass essential oil, and 10% lemongrass essential oil, respectively.

First, the pigeons were anesthetized by intramuscular injection of a mixture containing ketamine (10%, Alfasan, Woerden, Holland) and xylazine (2%, Alfasan, Woerden, Holland) at doses of 25 and 3 mg/kg, respectively. Then, the feathers on the back of the pigeon's neck were shaved, and the area was surgically scrubbed. Then, the skin of the spinal region of each pigeon was removed in full thickness and in a completely round shape with a diameter of 5 mm and a distance of 1 cm from each other using a skin punch. The wounds were treated daily for 14 days.

For the preparation of pistachio hull hydroalcoholic extract ointment, the maceration method was used. Pistachios of the species *P. vera* cv. *Kallehghuchi* was purchased from Kerman city and authenticated by the Department of Botany of the Amol University of Special Modern Technologies. The hulls were separated, washed, and dried in the shade at room temperature for one week. In the next step, the dried pistachio hulls were ground into a powder using a mill. The obtained powder was passed through a 60-mesh sieve. For extract preparation, 200 g of the powder was dissolved in one liter of ethanol and distilled water (70:30 v/v) and placed in a shaker at 150 rpm and 40°C for 24 hours. The mixture was then filtered through filter paper, and at least 90% of the solvent was removed using a rotary evaporator under vacuum. Finally, the extract was placed in an oven at 45°C for final concentration. The obtained material was stored in a freezer at –20°C until use (Moloudi *et al*., 2014; Naji *et al*., 2017). For treatments, ointments at concentrations of 5% and 10% were prepared as follows (Mohammadi *et al*., 2021). After determining the therapeutic doses, for the 5% ointment, 5 g of the extract was added to a 95 g base containing a mixture of Vaseline and Eucerin. For the 10% ointment, 10 g of the extract was added to a 90 g base containing a mixture of Vaseline and Eucerin. All stages of ointment preparation were carried out under sterile and aseptic conditions (Mohammadi *et al*., 2021).

The essential oil of lemongrass (LG) was prepared from the aerial portions of young leaves collected from Mirnia Plant Farm, located in Babol, Mazandaran Province, Iran. Botanical identification of the plant material was verified by the Sari Faculty of Agriculture and Natural Resources, Mazandaran Province. The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. Subsequently, gas chromatography was employed to characterize the chemical composition of the extracted oil (Table 1). Chemical profiling of the LG essential oil was performed using an Agilent 6890 gas chromatograph coupled with an Agilent Technologies 5973 inert mass selective detector. Separation was achieved on a capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature program began at 50°C and increased at a rate of 15°C/minute to 240°C, where it was maintained for 30 minutes. Samples were diluted in hexane prior to injection, and helium served as the carrier gas. A 1 µl injection volume was used with a split ratio of 20:1. The injector temperature was set at 300°C, while both the injector and detector were maintained at 280°C. Compound identification was accomplished by comparing retention times and mass spectra with authentic standards or published retention index (RI) data. Furthermore, the acquired mass spectra were matched against those available in the National Institute of Standards and Technology (NIST) library (Mirzakhani *et al*., 2024). Following determination of the appropriate therapeutic concentrations, LG ointments containing 5% and 10% essential oil were formulated. The 5% formulation was prepared by incorporating 5 g of essential oil into 95 g of an ointment base composed of Vaseline and Eucerin (Samin Co., Tehran, Iran). Similarly, the 10% formulation consisted of 10 g of essential oil blended with 90 g of the same base. All formulation procedures were performed under sterile conditions using aseptic techniques to ensure product quality.

Table 1. Chemical components of lemongrass (LG) essential oil.

No	Component[a]	Calculated RI[b]	Abundance (%) [c]
1	Myrcene	991	10.64
2	(Z)-β-Ocimene	1037	0.52
3	(Z)-β-Ocimene	1056	0.26
4	Linalool	1099	0.92
5	Citronellal	1149	0.34
6	Citronellol	1225	0.61
7	Neral d	1238	35.54
8	Geranial d	1266	49.38
9	Geranyl acetate	1379	0.34
	Total identified (%)	-	98.55

[a] Compounds were presented according to their order of elution on the HP-5MS capillary column.[b] Linear retention indices (RI) were experimentally determined on the HP-5MS column using a homologous series of C_8–C_{30} *n*-alkanes.[c] Relative percentage values represented the mean of three independent measurements, with the relative standard deviation (RSD) remaining below 10% for all compounds.[d] Neral and geranial were geometric isomers of citral; therefore, their combined content accounted for approximately 85% of the total essential oil composition in the present study (Mirzakhani *et al*., 2024).

The mRNA expression levels of IL-6, TNF-α, and IL-10 in pigeon skin tissue were determined by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was isolated from skin tissue samples using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. RNA concentration and purity were evaluated spectrophotometrically by measuring the absorbance ratio at 260/280 nm. To eliminate residual genomic DNA, 1 µg of total RNA was treated with DNase I (Fermentas, Lithuania) under RNase-free conditions. First-strand complementary DNA (cDNA) was synthesized from DNase-treated RNA using SuperScript III Reverse Transcriptase (Fermentas) and oligo(dT) primers by incubation at 42°C for 60 minutes, followed by enzyme inactivation at 70°C for 10 minutes. The synthesized cDNA was diluted (1:20) with nuclease-free water and used as the template for subsequent qPCR amplification. Real-time PCR was performed using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) on a Rotor-Gene 6000 Real-Time PCR System (Corbett Research, Australia). Each 20 µl reaction contained 10 µl of 2 × SYBR Green Master Mix, 0.8 µl of each primer pair (10 µM), 5 µl of diluted cDNA, and nuclease-free water to the final volume. Amplification was carried out under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 40 seconds. Following amplification, melting curve analysis was performed over a temperature range of 55–95°C with 0.5°C increments every 2 seconds to verify the specificity of the amplified products and exclude nonspecific amplification or primer-dimer formation. The relative mRNA expression levels of IL-6, TNF-α, and IL-10 were normalized against GAPDH as the endogenous reference gene and quantified using the 2^−ΔΔCt method (Mashayekhpour *et al*., 2025). All reactions were performed in triplicate, and the primer sequences used in this study were provided in Table 2.

Table 2. Primer sequences used in this study.

Target		Primer Sequences	Product size, bp
GAPDH	forward	5′-CCGGGCTGCTATTCTCTCTG-3′	141
	reverse	5′-TTCCCATTCTCGGCCTTGAC-3′	
IL-10	forward	5′-GACCAGCACCCTTCACC-3′	111
	reverse	5′-TTGCTCCTCTTCTCGCA-3′	
TNFα	forward	5′-GTGTTCAGTCCCTCCTCGTT-3′	230
	reverse	5′-TCAATCACAAGCAATGGGAGC-3′	
IL-6	forward	5′-GTGCAAAGCTCAAGTCCAA-3′	108
	reverse	5′-AATAGCGAACAGCCCTCAC-3′	

Statistical analyses were conducted using GraphPad Prism software (version X.X; GraphPad Software, San Diego, CA, USA). All data were expressed as mean ± standard error of the mean (SEM). Statistical differences between multiple experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant when the calculated *p* value was less than 0.05.

Results

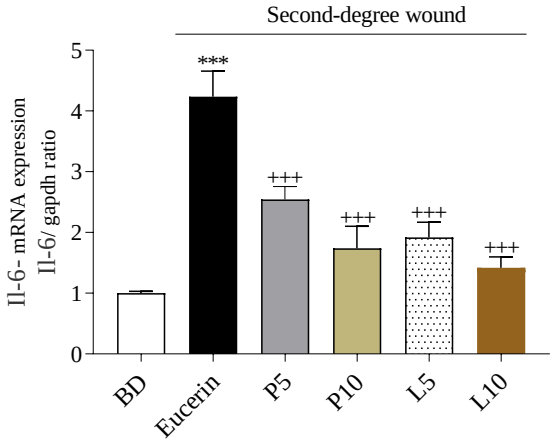
Vehicle control group (Eucerin) showed a significant increase in IL-6 expression compared to the untreated control group (BD), suggesting a strong pro-inflammatory response. P5, P10, L5,

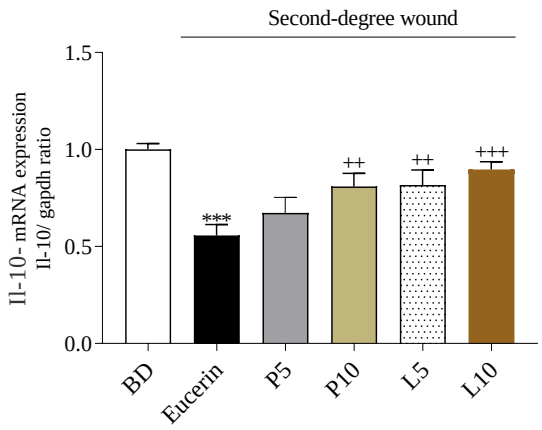
and L10 groups all showed reduced IL-6 levels compared to Eucerin, closer to BD, indicating a suppression of inflammation. Among them, P10 and L10 exhibited the lowest IL-6 levels, suggesting stronger anti-inflammatory effects.

Eucerin significantly decreased IL-10, an anti-inflammatory cytokine, compared to BD. This means Eucerin may impair anti-inflammatory regulation. Conversely, P5, P10, L5, and L10 groups increased IL-10 expression compared to Eucerin, some even reaching or surpassing BD levels. L10 appeared to restore IL-10 expression most effectively, followed closely by L5 and P10.

Similar to IL-6, Eucerin caused a significant increase in TNF-α expression, reflecting a heightened inflammatory state. P5, P10, L5, and L10 again reduced TNF-α expression, aligning closer to BD. The lowest levels were observed in P10 and L10, showing strong anti-inflammatory potential (Fig. 1).

Eucerin consistently elevated pro-inflammatory cytokines (IL-6, TNF-α) while suppressing the anti-inflammatory cytokine (IL-10). In contrast, P and L treatments (especially P10 and L10) not only reduced IL-6 and TNF-α but also enhanced IL-10, suggesting a balanced immune response that could favor wound healing. P10 and L10 seem to provide the most promising results in terms of reducing inflammation and enhancing anti-inflammatory signaling.





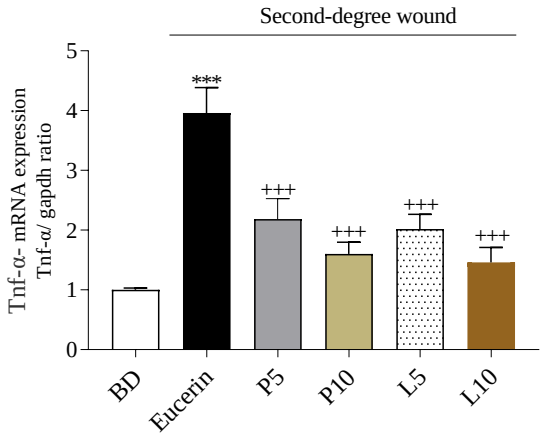


Fig. 1. Effects of pistachio and lemongrass formulations on inflammatory cytokine gene expression during wound healing. Relative mRNA expression levels of IL-6, IL-10, and TNF-α were quantified by qRT-PCR and normalized to β-actin. The experimental groups consisted of untreated control (BD), vehicle control (Eucerin), pistachio 5% (P5), pistachio 10% (P10), lemongrass 5% (L5), and lemongrass 10% (L10).

Discussion

The present study demonstrated that topical administration of hydroalcoholic pistachio hull extract and lemongrass essential oil significantly accelerated excisional wound healing in pigeons. Treatment with both compounds downregulated the expression of the pro-inflammatory cytokines IL-6 and TNF-α and increased IL-10 expression, indicating that modulation of the inflammatory response is an important mechanism contributing to enhanced tissue repair.

The beneficial effect of pistachio hull extract may be attributed to its rich content of phenolic

compounds, flavonoids, and tannins, which possess strong antioxidant and anti-inflammatory activities (Goli *et al*., 2005) first demonstrated that pistachio hull contains exceptionally high concentrations of phenolic antioxidants, while Rajaei *et al*. (2010) reported significant antimicrobial and antioxidant properties of pistachio green hull extract. These biological activities probably reduced oxidative stress and microbial burden within the wound environment, thereby facilitating tissue regeneration. More recently, Sarkhail *et al*., (2020) identified 3-epimasticadienolic acid as one of the principal wound-healing compounds of *Pistacia vera* hull and demonstrated that this triterpenoid significantly enhanced fibroblast migration while suppressing IL-6 and TNF-α expression. Furthermore, a subsequent study by the same group showed that anacardic acid isolated from pistachio hulls also promoted fibroblast migration and wound closure with minimal cytotoxicity, supporting the traditional use of pistachio hulls as a wound-healing agent.

Although few veterinary studies have evaluated lemongrass essential oil effects on cutaneous wound healing, its therapeutic activity has been attributed to citral, the major constituent of the essential oil, which possesses antioxidant, antimicrobial, and anti-inflammatory properties. The protective effects of lemongrass essential oil observed in the present study are also consistent with the findings of Mirzakhani *et al*., (2024), who demonstrated that lemongrass essential oil reduced oxidative tissue damage and inflammatory lesions in a murine model of intestinal injury. Collectively, these findings indicated that the biological activity of lemongrass extends beyond gastrointestinal protection and may also facilitate cutaneous tissue repair through regulation of inflammation and oxidative stress.

Modulation of inflammatory cytokines represented the major findings of this study. Appropriate regulation of inflammation is essential for normal wound healing because prolonged elevation of pro-inflammatory cytokines delays epithelialization and extracellular matrix remodeling (Cedillo-Cortezano *et al*., 2024). In the present study, both herbal preparations significantly decreased IL-6 and TNF-α expression while simultaneously increasing IL-10 expression. This cytokine profile indicated earlier resolution of inflammation and progression toward the proliferative phase of healing.

The molecular findings are in agreement with previous reports describing the anti-inflammatory properties of *Pistacia* species (Bozorgi *et al*., 2024). Experimental studies have shown that bioactive compounds isolated from pistachio hulls suppress inflammatory signaling while enhancing fibroblast proliferation and migration. Likewise, recent reviews have emphasized that phenolic-rich medicinal plants promote wound healing through simultaneous antioxidant, antimicrobial, and immunomodulatory activities rather than through a single mechanism (Pathak and Mazumder, 2024). Therefore, the reduction of IL-6 and TNF-α together with the increase of IL-10 in the present study provides a plausible molecular explanation for the accelerated wound healing during macroscopic observation.

The 10% formulations consistently produced more favorable cytokine profiles than the corresponding 5% formulations. These observations support the use of higher concentrations of both pistachio hull extract and lemongrass essential oil for topical wound management.

In conclusion, topical application of hydroalcoholic pistachio hull extract and lemongrass essential oil significantly accelerated excisional wound healing in pigeons. Faster wound healing was accompanied by suppression of IL-6 and TNF-α and increased IL-10 expression, indicating that regulation of inflammation plays a central role in their wound-healing activity. Overall, the 10% formulations exhibited the greatest therapeutic efficacy, suggesting that both pistachio hull extract and lemongrass essential oil may represent promising natural topical agents for the treatment of cutaneous wounds in avian medicine.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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