JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLE

J Res Vet Med. 2023: 42 (2) 54-64
DOI:10.30782/jrvm.1217557

Effects of *Salvia Tomentosa* Ointment on Dermal Wound Healing in a Rabbit Model

**Mehmet Mustafa DEMIR[1], Volkan İPEK[2,*], Hidayet TUTUN[3]
Harun ÇINAR[4], Leyla Elif ÖZGÜ AYGÖZER[2]**

1Burdur Mehmet Akif Ersoy University, Health Sciences Institute, Burdur, Turkey,
2Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, Burdur, Turkey,
3Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Burdur, Turkey,
4Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Surgery, Burdur, Turkey

Received 11-12-2022 Accepted 13-10-2023

Abstract

This study aimed to evaluate the wound healing activity of an ointment containing the hydroethanolic extract of *Salvia Tomentosa* (*S. tomentosa*) in a dermal wound model using rabbits. Ointments with concentrations of 2.5% (w/v) and 5% (w/v) were prepared using the hydroethanolic extracts of *S. tomentosa*. An *in vivo* excisional wound model was embloyed with New Zealand rabbits (n = 35) to assess the wound healing activity of these ointments. Rabbits were randomly divided into five experimental groups: negative (received no treatment), vehicle (received only the ointment vehicle), positive control (treated with Fitokrem®), and those treated with 2.5% (w/v) and 5% (w/v) *S. tomentosa* ointments. Wound diameters were measured using calipers on the 4th, 8th, and 14th days, and histopathological examinations were conducted on the 3rd, 7th, and 14th days of treatment. The total phenolic content of the *S. tomentosa* hydroethanolic extract was determined to be 61.52 ± 2.33 µg of Gallic Acid Equivalents per mg of dry plant material. The total phenolic content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the hydroethanolic extract of Salvia tomentosa were determined spectrophotometrically. In terms of wound closure, both 2.5% *and5%S. tomentosaointmentsdemonstratedwoundhealingactivity comparableto thatofFitokrem®. Inconclusion, S. tomentosaexibits* a positive effect on dermal wound healing in rabbits.

Keywords: Rabbit, radical scavenging activity, *Salvia tomentosa*, total phenolic content, wound healing

Introduction

Wound healing is a multifaceted and dynamic process that engages multiple cell types, an array of cytokines, and mediators. This intricate process results in the substitution of dead structures and tissue layers with new ones.[1,2] Various factors, such as presence of infection, immunity, and nutrition, influence the wound healing process. Consequently, adequate wound care is paramount to reduce infections and enhance healing.[3] Wound healing presents challenges due to the use of synthetic drugs. These drugs often come with high cost and potential side effects, including antibacterial resistance, reduced effectiveness, and allergic reactions. Thus, there is a pressing need for alternative agents that can promote swift and high-quality wound healing.[4,5]

For centuries, plants have been utilized to address and prevent a plethora of diseases, including malaria, stomach ulcers, wounds, sores, hemorrhoids, cancer, toothaches, and infections, in numerous countries worldwide.[6–10] Derived from various plant parts such as leaves, fruit, stems, roots and bark, herbal remedies are crafted into creams, lotions, gels, solvent extracts, or ointments. These formulations, known for their antimicrobial and antioxidant activities, are then used for skincare.[11–13] It's well-documented that ointments infused with plant extracts in various proportions enhance wound healing across different wound types.[14–16] Globally, a vast array of plant species from families such as Fabaceae, Combretaceae, Asteraceae, and Lamiaceae have been harnessed as wound healing solutions in traditional medicine.[17–20]

* Corresponding author: Volkan Ipek volkanipek@mehmetakif.edu.tr, Gsm: +90 554 614 2701, Fax: +90 248 213 20 01

In Turkish fol medicine, *Salvia Tomentosa* is a popular choice for wound healing, attributed to its cost-effectiveness and accessibility. Boasting over 700 species, the Salvia genus, part of the Lamiaceae (Labiatae) family, thrives in the tropical and subtropical regions, notably in the Mediterranean and Central Europe.[21] Turkey is home to 92 Salvia species, with 44 being endemic to the region.[22] *S. tomentosa* is predominantly found the Mediterranean and Aegean regions of Turkey. It's not only consumed as an herbal tea but it also a traditional remedy for ailments such as abdominal pain, flatulent dyspepsia, laryngitis, oral mucosal inflammation, pharyngitis, tongue inflammation, and wounds.[17,21,23]

Research on the phytochemicals and pharmacological attributes of Turkish *S. tomentosa* remain limited. However, various extracts of *S. tomentosa* have displayed both antibacterial[17,24] and antifungal prowess.[25] Different solvent extracts of *S. tomentosa* have showcased notable antioxidant activity in wounds.[25–29] The plant's rich secondary metabolite content, including phenolics and terpenoids, is believed to underpin its antimicrobial and antioxidant properties. Prominent flavonoids of *S. tomentosa* include rosmarinic acid, caffeic acid, morin, p-coumaric acid, and myricetin.[17,24,26,30] The antioxidant and antibacterial efficacy of *S. tomentosa* in wounds could be instrumental in shielding tissues from infections. To the best of the authors' knowledge, no studies have yet confirmed its wound healing capabilities. This research, therefore, delves into the wound healing potential of an ointment containing the hydroalcoholic extract of *S. tomentosa* in a rabbit dermal wound model.

Material and Methods

Plant Material

The aerial parts of *S. tomentosa* were harvested from the Çeltikçi district (GPS Coordinates: 37°35'50.2" N, 30° 24'08.3" E) in the Burdur province of Türkiye in July 2020. These were then dried in the shade at room temperature (25 °C). The plant sample was authenticated, and reference specimens were archived in the Konya Herbarium with the voucher accession number 25899 (Fig 1).

Figure 1. Herbarium archive photo of *Salvia tomentosa* Miller (Knya herb No: 25899).

Preparation of Hydroethanolic Extraction

The dried plant sample was pulverized using an electric blender. A 100 g portion of the powdered plant was suspended in 500 ml of a water/ethanol (30–70% v/v) mixture. Extraction proceeded at 180 rpm at room temperature for 8 hours in a Thermo shaker, ensuring complete solution homogeneity.[31] Subsequently, the solution underwent centrifugation at 5,000 rpm using a centrifuge (Hettich Rotina 38R) for 15 minutes. Filtration was carried out employing Whatman No.1 filter paper. The obtained filtrate was then concentrated via a rotary evaporator (RV10, IKA ®, Germany) at 40°C. The resultant concentrated extract was lyophilized using a freeze dryer (Martin Christ, Alpha 1-2LD plus, Germany) and stored in a sealed bottle in the dark at 4°C.

Ointment Preparation

An ointment was formulated using the lyophilized extract. Table 1 provides a detailed breakdown of the ointment's constituents. In brief, the components of Phase B were added to a beaker and heated to between 70-80 °C. Meanwhile, the Phase A components were melted in a separate beaker by heating them to the same temperature range. The molten phases A and B were combined, mixing at a rate of 600-800 rpm using a laboratory mixer. For Phase C, the *S. tomentosa* extract and polyethylene glycol were dissolved in 15-20 ml of distilled water (constituting a portion of the ointment's total water content) and were homogenized. Once the combined temperature of phases A and B dropped below 40 °C, C phase portion was introduced. The remaining Phase C components were then incorporated, and stirring continued until the mixture reached room temperature.

Table 1. *Salvia tomentosa* ointment content.

	Substances	Control	2.5% extract	5% extract
	Glyceryl stearate and Peg-100 stearate	6.0	6.0	6.0
	Pentaerythrityl distearate	2.5	2.5	2.5
	Myristyl myristate	2.0	2.0	2.0
A	Hydrogenated vegetable glycerides	3.0	3.0	3.0
	Caprylic/capric triglyceride	4.0	4.0	4.0
	Coco caprylate	2.0	2.0	2.0
	Hexyldecanol and hexyldecyllaurate	2.5	2.5	2.5
	Cetearyl alcohol	3.0	3.0	3.0
B	Water	65.8	63.3	60.8
	Glycerin	7.0	7.0	7.0
C	Dehydroacetic acid and benzyl alcohol	0.2	0.2	0.2
	Salvia tomentosa extract	-	2.5	5.0
	Peg-8	2.0	2.0	2.0

PEG-100: Polyethylene glycol monostearate; Peg-8: Polyethylene glycol

Determination of Total Phenolic Content

The total phenolic content (TPC) of the *Salvia Tomentosa* crude extract was gauged using the Folin – Ciocalteu method. This was based on the protocol proposed by Pawar and Dasgupta[32], with results expressed in µg Gallic Acid Equivalents (GAE) per mg of dry plant material. Briefly, gallic acid standart solutions, ranging from 3.12–200 µg/ml (prepared through two-serial dilutions in ethanol from Merck, Germany), were set. A 200 µl aliquot of the filtered extract (1 mg/ml in ethanol) or the standard solution was combined with 400 µl of distilled water. To this mixture, 200 µl of the 10% Follin – Cicocalteu's phenol reagent (diluted with distilled water) was added. Following a 5-minute interval, 200 µl of a 1 M sodium carbonate solution was introduced. The solution was then incubated at room temperature for 30 minutes, shielded from light. Post incubation, 300 µl of the mixture was transferred to a 96-well plate, using ethanol as the reference blank. Absorbance was then taken at 750 nm using a microplate spectrophotometer (Multiskan Go, Thermo Scientific).

Radical Scavenging Assay

The free radical scavenging activity of 2,2 – Diphenyl – 1 – picrylhydrazyl (DPPH) was assessed following a previously established procedure.[33] Briefly, 50 µL of extract at varying concentrations (ranging from 10–1000 ug/ml) in ethanol was introduced to 150 µL of a 200 µM methanolic DPPH solution in a 96-well plate. The reaction blend was incubated for 30 minutes at room temperature in a dark setting. Absolute methanol served as the reagent blank. Absorbance readings were recorded at 517 nm using a Microplate Reader (Multiskan Go, Thermo Scientific). Ascorbic acid acted as benchmark control. The DPPH radical scavenging activity (%) was derived using the formula: DPPH scavenging activity (%) = [(Ac – As) / Ac] × 100, where Ac denotes the absorbance of control (DPPH + Methanol without the sample] and As presents the sample's absorbance (DPPH + Extract). The 50% inhibitory concentration (IC50) was deduced from the plot mapping the inhibition percentage against extract concentration (µg/ml).

Wound Healing Activity

Animals

A total of 35 adult male New Zealand rabbits, approximately one year old and averaging a body weight of 3.5 kg, were chosen to establish the excision wound model. The animals' care, feeding, and biopsy procedures were overseen at the Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center. Rabbits were individually housed in standard cages measuring 45 × 40 × 50 cm. They were maintained under standard conditions, which included a 12:12-hour light-dark cycle, 50-70% humidity, and a temperature range of 25°C ± 3°C). They were provided unrestricted access to standard feed and water. The study adhered to the guidelines of the European Community Council Directive (2010/63/EU). The Burdur Mehmet Akif Ersoy University Rectorate Animal Experiments Local Ethics Committee approved the study, as reflected in the decision dated January 15, 2020, bearing the number 610.

Excision Wound Model

Our methodology for creating the excision wound model in rabbits was adapted from the protocol established by Huang et al.,[34]. Rabbits were anesthetized using a combination of ketamine and xylazine (40 mg/kg ketamine; 20 mg/kg xylazine). While under anesthesia, each rabbit's dorsal skin was shaved using an electric clipper and subsequently sanitized with a topical povidone-iodine. Four full-thickness wounds, each measuring 1.5 cm × 1.5 cm, were made on both the left and right sides of the midline on each animal's back. Three biopsies were taken from three distinct wounds on each animal. At the study's conclusion, a fourth wound was left unbiopsied to assess the degree of wound closure.

Treatment Schedule

Rabbits were categorized into five groups, each comprising seven individuals. Group 1 served as the negative control, receiving no treatment. Group 2, the vehicle group, was administered the vehicle ointment. Group 3, designated as the positive control, was treated with Fitokrem®, a commercial ointment formulated for wound healing. Groups 4 and 5 were respectively treated with ointments that contained 2.5% and 5% *S. tomentosa* extract. Treatments were

applied topically, with ointments smeared on each rabbit's wound area daily. Biopsies for histopathological evaluation were obtained on the 3rd, 7th, and 14th days under anesthesia induced by xylazine and ketamine. The right caudal wound's complete healing facilitated the assessment of the wound closure level. On day 14, the diameters of the right caudal wounds were measured using calipers. During the study's course, due to complications, deteriorating health conditions, or mortality, two animals from the control group, two from the Fito group, one from the vehicle group, and two from the 5% *S. tomentosa* group were excluded. Wound areas were also delineated with a caliper immediately post-wound induction. The recovery rate (percentage of wound closure) was calculated using the following formula: Revovery rate (%) = [(wound diameter on day 0 – wound diameter on day 14) / wound diameter on day 0] x 100.35 The quantitative data and conclusions drawn from this research were primarily based on caliper measurements, ensuring the accuracy and reliability of our findings.

Histopathology

Post fixation in a 10% neutral buffered formaldehyde solution, 4 µm thick cut sections were taken from the biopsy samples, embedded in paraffin and stained with hematoxylin and eosin (H & E). Within the wounded region, histopathologists evaluated aspects like inflammatory cell infiltrations (both acute and chronic), angiogenesis, the amount of granulation tissue, and fibroblast maturation levels. Angiogenesis was scored as 0 = absent, 1 = mild (less than 5 vessels in 1 high magnification), 2 = moderate (6-10 vessels in 1 high magnification), and 3 = evident (more than 10 vessels in 1 high magnification). Inflammation was scored as 1 = very mild (0-25 cells in 3 high magnification), 2 = mild (25-50 cells in 3 high magnification), 3 = medium (50-75 cells in 3 high magnification), and 4 = (75-100 cells in 3 high magnification). The amount of fibrosis and fibroblast maturation levels were also scored as 0 = absent, 1 = mild, 2 = moderate, and 3 = significant. The scoring approach was consistent with prior literature.[36,37] Fibroblast maturation was assessed based on the cellular morphology and arrangement in the tissue. Mature fibroblasts were identified by their elongated spindle shape, organized arrangement in parallel arrays, and reduced cellular density compared to the more proliferative zones of granulation tissue. Additionally, the presence of a well-developed extracellular matrix, indicative of active collagen production and deposition by mature fibroblasts, served as a key criterion for assessing fibroblast maturity.[38] Group comparisons were executed distinctly for each biopsy timeline.

Statistics

Statistical evaluations was performed using the Minitab 16 software package (Version 16.1.1). The Ryan-Joiner test was employed to assess data distribution's normality. Group comparisons were conducted using One-Way ANOVA coupled with a Posthoc Tukey test. The significance threshold was set at 0.05.

Results

Total Phenolic Content and Radical Scavenging Activity

The hydroethanolic extract of *S . t omentosa* exhibited a TPC of 61.52 ± 2.33 µg of GAE/mg of dry plant material (Table 2). Using the DPPH method, we assessed the antioxidant capacities the extract. The IC50 values for ascorbic acid and the extract stood at 14.45 ± 1.28 and 293.23 ± 5.43 µg/mL, respectively (Table 2). Notably, the extract demonstrated low antiradical capacity compared to ascorbic acid.

Table 2. DPPH radical scavenging activity and TPC of *Salvia tomentosa* hydroethanolic extracts.

Sample	IC_{50} values (µg/mL)	TPC (µg of GAE/mg)
Ascorbic acid	14.45 ± 1.28	-
S. Tomentosa extract	293.23 ± 5.43	61.52 ± 2.33

Values are presented as mean ± Standard deviation of triplicate experiments.

DPPH: 2,2 – diphenyl – 1 – picrylhydrazyl; TPC: Total phenolic content

Table 3. Comparison between groups in terms of wound closure levels.

Group	Wound closure rates (%)
	14th
Group 1 (Negative control)	35.20 ± 8.00[b]
Group 2 (Vehicle control)	31.33 ± 6.65[b]
Group 3 (Positive control, Fitokrem®)	72.80 ± 6.14[a]
Group 4 (Ointment containing 2.5% *S. tomentosa* extract)	68.43 ± 5.32[a]
Group 5 (Ointment containing 5% *S. tomentosa* extract)	72.60 ± 13.80[a]
P value	0.001

a, b: The statistical difference between the means with different letters in the same row is significant

Macroscopic Observations

Wound closure rates in the Fitokrem®, *S. tomentosa* 2.5% (ST-2.5) and 5% (ST-5) groups significantly outperformed those in the negative control and vehicle groups (p = 0.001) (Table 3). Although there was no statistical difference between the extracts and Fitokrem®, full wound closure was observed exclusively in 2 animals from the ST-5 group (Fig 2).

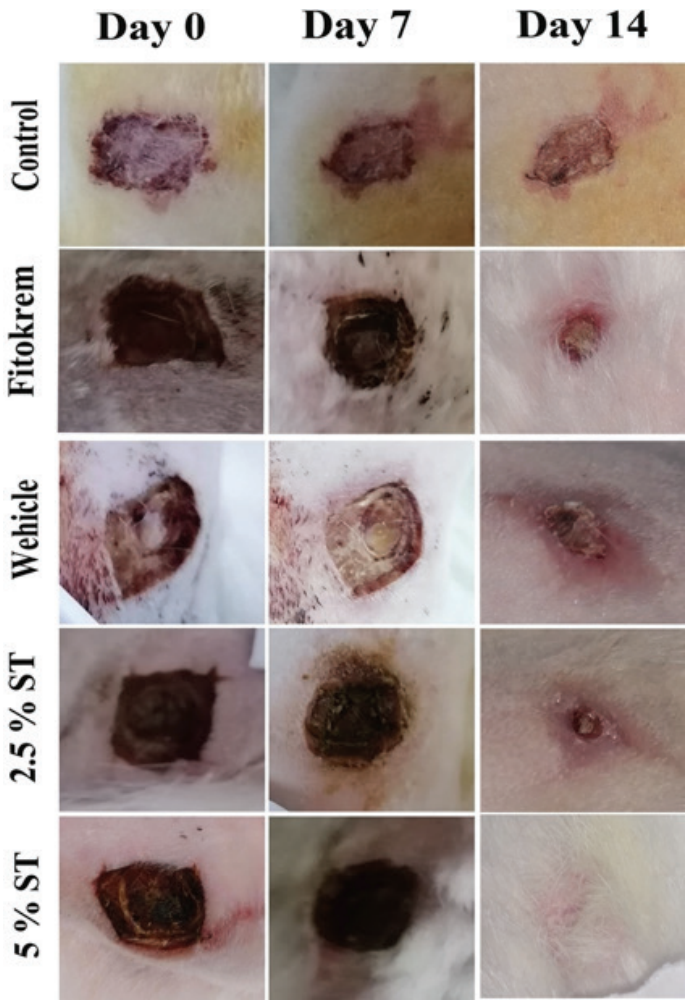

Figure 2. Wound closure levels in groups at 0, 7 and 14 days.

Histopathological Findings

Throughout all groups and as time progressed, there was a noticeable increase in angiogenesis, fibrosis, and fibroblast maturation, while inflammation showed a consistent decline.

On the 3rd day, every group displayed extensive necrotic tissue in the wound area, accompanied by pronounced neutrophil infiltrations at the wound edges. This was particularly evident in Fig 3A-3E. Angiogenesis was observed to be mild to moderate, especially proximate to the necrotic tissue. The control group stood out as it didn't show any signs of fibrosis. On the other hand, mild fibrosis was identified in certain animals from the vehicle, Fitokrem, and ST-5 groups.

By the 7th day, the control group had mild to moderate dermal fibrosis, some regeneration of the epidermis, and an observable uptick in angiogenesis (Fig 3F). The vehicle group showed signs of partial epidermal regeneration, mild fibrosis in the dermis, and a notable increase in both inflammation and angiogenesis (Fig 3G). The Fitokrem group, on the other hand, exhibited extensive epidermal regeneration with enhanced angiogenesis and fibrosis (Fig 3H). The ST-2.5 group was characterized by evident dermal fibrosis, extensive regeneration of the epidermis, and a decline in inflammation (Fig 3I). Lastly, the ST-5 group presented significant fibrosis in the dermis, wide-ranging

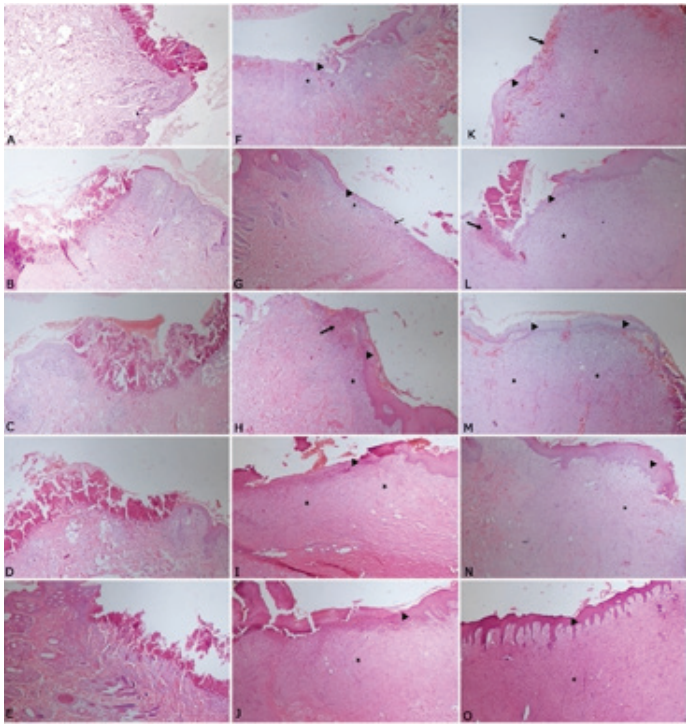

Figure 3. On the 3rd day, the wound edge appearances were observed in the Control (A), Vehicle (B), Fitokrem (C), ST-2.5 (D), and ST-5 (E) groups. By the 7th day, the Control group exhibited partial epithelial regeneration (arrow head) and fibroblast proliferation (star) in the dermis (F). The vehicle group showed partial re-epithelialization (arrow head), fibrosis (star) and neutrophil infiltrations (arrow) (G). The Fitokrem group had an extensive re-epithelialization area (arrow head) with dermal fibrosis and dense neutrophil infiltrations (star) at the wound margin (H). The ST-2.5 group displayed re-epithelialization (arrow head) and dermal fibrosis (stars) (I), while the ST-5 group demonstrated pronounced fibrosis (star) in the dermis alongside re-epithelialization (J). On the 14th day, the control group had a partial re-epithelialization area (arrow head), dense dermal fibrosis (star), hemorrhage, and inflammatory cell infiltrations in the healing region (arrow) (K). The Vehicle group was marked by re-epithelialization (arrow head), dermal fibrosis (arrow head), and inflammatory cell infiltrations (arrow) (L). The fitokrem group showed maturation from a large re-epithelialization area (arrow head) and had substantial fibrosis in the dermis (stars) with multiple new vessel formations (M). The ST-2.5 group revealed a large re-epithelialization area (arrow head) and widespread dermal fibrosis (star) (N). Lastly, the ST-5 group presented with complete re-epithelialization (arrow head) and a mature dermal healing area (arrow head) (O). H&E. Bars: 50 μm.

regeneration of the epidermis, and varying levels of inflammation across animals (Fig 3J).

Moving to the findings on the 14th day, the control group showed intense fibrosis in the dermis, partial regeneration of the epidermis, and a general increase in angiogenesis

(Fig 3K). The vehicle group displayed extensive regeneration of the epidermis, marked proliferation of fibrocytes in the dermis, and a clear emphasis on matured connective tissue (Fig 3L). The Fitokrem group had extensive fibrosis in the dermis with an observable rise in the formation of new vessels (Fig 3M). The ST-2.5 group displayed a wide area of epidermal regeneration and pronounced dermal fibrosis (Fig 3N). Notably, in the ST-5 group, two animals exhibited full epidermal regeneration accompanied by well-matured dermal fibrous tissue (Fig 3O).

In terms of angiogenesis, the 3rd day presented no significant differences across groups. By the 7th day, however, the TS-2.5 group was found to surpass the control group in angiogenesis ($p = 0.028$). By the 14th day, all other groups showed no significant differences in comparison to the control. When considering acute inflammation, the results from the 3rd and 7th day biopsies were consistent across all groups. However, by the 14th day, there was a marked decrease in neutrophil leukocytes in the ST-2.5 and ST-5 groups, especially when compared to the control and vehicle groups ($p = 0.003$). Regarding fibrosis volume and fibroblast maturation, results from the 3rd day biopsies were consistent across all groups. However, by the 7th day, there was an observed increase in fibrosis for the ST-2.5 and Fitokrem groups in comparison to the vehicle group ($p = 0.005$). The findings from the 14th day showed no major differences across groups. In terms of fibroblast maturation, there was no significant difference between the groups in the 3rd day biopsies, but the vehicle group showed significantly lower maturation in the 7th day biopsies compared to all other groups ($p = 0.000$).For a comprehensive understanding, detailed statistical analyses of these histopathological parameters are provided in Table 4.

Table 4. Statistical comparison between groups in terms of histopathological parameters.

Day	Group	Angiogenesis	Acute Inflammation	Fibrosis	Fibroblast Maturation
3	**Control**	1.00 ± 0.00^{a}	3.00 ± 0.63^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Vehicle	1.16 ± 0.16^{a}	2.33 ± 0.55^{a}	0.50 ± 0.22^{a}	0.00 ± 0.00^{a}
	Fito	1.80 ± 0.49^{a}	3.80 ± 0.20^{a}	0.80 ± 0.37^{a}	0.20 ± 0.20^{a}
	***S. tomentosa* 2.5%**	1.85 ± 0.34^{a}	3.42 ± 0.36^{a}	1.00 ± 0.30^{a}	0.57 ± 0.29^{a}
	***S. tomentosa* 5%**	2.20 ± 0.37^{a}	3.00 ± 0.54^{a}	0.80 ± 0.37^{a}	0.40 ± 0.40^{a}
	P value	0.086	0.294	0.179	0.361
7	**Control**	2.20 ± 0.37^{b}	2.60 ± 0.40^{a}	1.40 ± 0.24^{ab}	1.80 ± 0.20^{a}
	Vehicle	2.83 ± 0.30^{ab}	2.83 ± 0.40^{a}	0.83 ± 0.16^{b}	0.66 ± 0.21^{b}
	Fito	2.60 ± 0.24^{ab}	2.60 ± 0.51^{a}	2.20 ± 0.37^{a}	2.00 ± 0.00^{a}
	***S. tomentosa* 2.5%**	3.42 ± 0.20^{a}	2.28 ± 0.36^{a}	2.28 ± 0.28^{a}	2.00 ± 0.00^{a}
	***S. tomentosa* 5%**	2.80 ± 0.37^{ab}	2.40 ± 0.24^{a}	2.00 ± 0.31^{ab}	2.40 ± 0.24^{a}
	P value	**0.028**	0.862	**0.005**	**0.000**
14	**Control**	3.00 ± 0.54^{a}	2.40 ± 0.51^{a}	2.40 ± 0.24^{a}	2.00 ± 0.00^{a}
	Vehicle	2.50 ± 0.22^{a}	2.50 ± 0.34^{a}	2.16 ± 0.30^{a}	1.83 ± 0.16^{a}
	Fito	3.20 ± 0.20^{a}	1.40 ± 0.51^{ab}	3.00 ± 0.00^{a}	2.40 ± 0.24^{a}
	***S. tomentosa* 2.5%**	3.14 ± 0.26^{a}	0.85 ± 0.26^{b}	2.71 ± 0.18^{a}	2.42 ± 0.20^{a}
	***S. tomentosa* 5%**	3.20 ± 0.37^{a}	0.60 ± 0.24^{b}	2.60 ± 0.24^{a}	2.40 ± 0.24^{a}
	P value	0.513	**0.003**	0.146	0.120

a, b: The statistical difference between the means with different letters in the same row is significant.

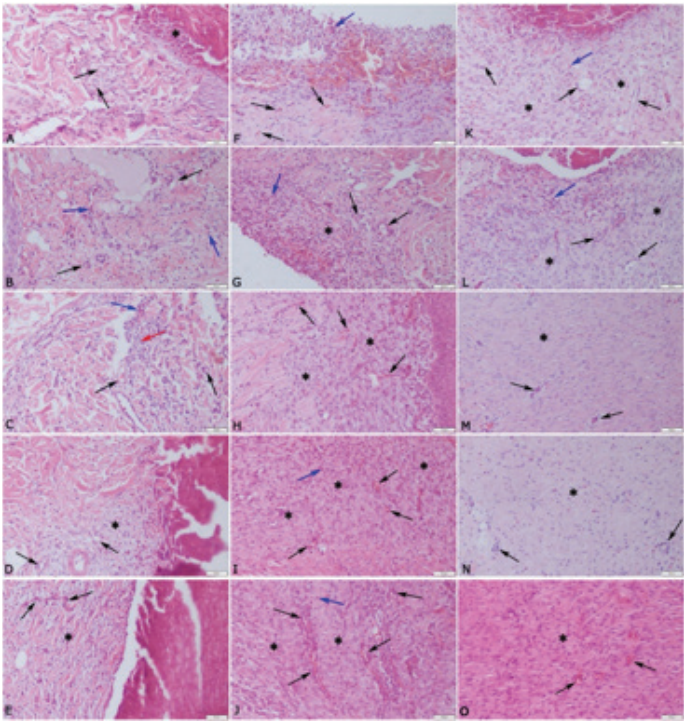

Figure 4. On the 3rd day, the Control Group (A) exhibited pronounced neutrophil infiltration (indicated by stars) and neovascularization (arrows), the Vehicle Group (B) showed neutrophil infiltrations (blue arrows) and vascular formations (arrows), the Fito Group (C) had dermal fibrosis, neutrophil (blue arrow), mononuclear cell infiltrations (red arrow), and neovascularization (arrows), the ST-2.5 Group (D) displayed fibroblast proliferation at the wound edge (stars) and neovascularization (arrows), and the ST-5 Group (E) demonstrated intense fibroblast proliferation (stars) and neovascularization (arrows). By the 7th day, the Control Group (F) presented fibroblast proliferation, inflammatory cell infiltrations (blue arrow), hemorrhage, and neovascularization (arrows) in the healing zone, the Vehicle Group (G) featured fibrosis in the healing zone (stars), neovascularization (arrows), and neutrophil infiltrations (blue arrow), the Fito Group (H) highlighted dermal fibrosis (stars) and neovascularization (arrows) in the healing area, the ST-2.5 Group (I) portrayed inflammatory cells in the dermis, neovascularization (arrows), and mature fibrous tissue (stars) in the healing zone, and the ST-5 Group (J) indicated dermal fibrosis (stars), neovascularization (arrows), and sparse inflammatory cell infiltrations (blue arrow). On the 14th day, the Control Group (K) displayed ongoing healing with dermal fibrosis featuring angiogenesis (arrows) and scattered inflammatory cell infiltrations (blue arrow), the Vehicle Group (L) showed fibrosis area with neovascularization (arrows) and neutrophil infiltrations (blue arrow) in the healing zone, the Fito Group (M) revealed advanced mature connective tissue area (star) featuring neovascularization (arrows), the ST-2.5 Group (N) exhibited advanced mature connective tissue area (star) with neovascularization (arrows), and the ST-5 Group (O) presented a well-developed dermal fibrosis area (star) with neovascularization (arrows). H&E. Bars: 50 µm.

Discussion

Throughout history, plants have been employed as therapeutic agents for wounds, attributed to their active compounds known for antioxidant and antimicrobial properties.[39–41] *Salvia Tomentosa*, a mainstay in Turkish folk medicine, is utilized for various ailments including swollen dyspepsia, laryngitis, pharyngitis, stomatitis, gingivitis, glossitis, hyperhidrosis, and galactorrhea.[42] The antioxidant potential of this plant largely due to the substantial precence of phenolic compounds.[43,44] Typically, there is a discernible correlation between a plant's antioxidant activity and it's total phenolic content.[30,45] The TPC value observed in our study (61.52 ± 2.33 µg of GAE/mg) aligns with previously reported values ranging from 10 to 275 µg GAE/mg for S. tomentosa extracted with various solvents.[25,30,46] Furthermore, the radical scavenging activity of our extract mirrored that of *S. tomentosa* extracts derived through different solvents in other studies.[30,47]

Phenolic compounds, representing a significant class of plant secondary metabolites, act as primary antioxidants and free radical scavengers.[48] Additionally, many of these phenolics possess direct antimicrobial effects.[49,50] Chronic oxidative stress is instrumental in the pathogenesis of impaired wound healing and scar development.[51] Several studies underscore the potential of phenolic compounds with robust antioxidant activities in accelerating wound healing. They achieve this by modulating inflammatory markers, reducing oxidative stress, and curtailing microbial growth.[52–54] In our study, the observed positive effects of *S. tomentosa* on wound healing can be attributed to its phenolic compound content and radical scavenging activity.

For our research, we utilized 2.5% and 5% hydroethanolic extracts of *S. tomentosa*. We observed that the macroscopic wound closure efficiency was notably higher in the Fitokrem and extract groups compared to the control and vehicle groups. Karimzadeh and Farahpour[55] investigated the effectiveness of *S. officinalis* on rat wounds and evaluated 1%, 3%, and 5% hydroethanolic extracts. Their findings emphasized the particularly beneficial impact of the 5% extract on wound healing. Similarly, Güzel et al.[37] reported significant wound closure levels using both Salvia extract and Fitokrem compared to controls when researching ethanol extracts of *S. kronenburgii* and *S. euphratica* in diabetic rat wounds. Pintapagung et al.[56] reported that a 4% n-hexane extract of *S. hispanica* positively impacted wound contraction in diabetic mice. Irmak et al.[57] assessed the efficiacy of orally administered *S. miltiorrhiza* in a post-burn wound healing model using rats. Their findings indicated that the extract not only reduced necrotic tissue in burn wounds, but also enhanced tissue perfusion and neovascularization. In another study, Salimikia et al.[58] observed that methanolic extracts of *S. multicaulis*, at concentrations of 5%, 10%, and 20%, facilitated wound healing in rats. Süntar et al.[59] investigated the potential of 1% ethanol extracts from *S. cryptantha* and *S. cyanescens* on wound models in both mice and rats, determining that *S. cryptantha particularly* contributed to wound healing. Consistently, in our research, the hydroethanolic extract of *S. tomentosa* showcased positive outcomes in macroscopic wound closure, echoing the effects seen in other Salvia species.

One essential factor for effective wound healing is swift re-epithelialization.[60,61] Güzel et al.[37] histopathologically identified enhanced epithelial regeneration in Fitokrem and all Salvia extracts compared to the control and vehicle groups. Due to the limitations of our biopsy samples, we couldn't score and compare re-epithelization levels comprehensively. Nevertheless, based on macroscopic observations, only the ST-5 group exhibited complete epithelialization. In contrast, all other groups showed partial re-epithelialization overlaying the dermal granulation tissue to varying extents.

Vascular endothelial cells are drawn to the wound site under the influence of macrophages.[62–64] Neovasculature is pivotal for epithelial cells to migrate from the wound's edges to its center.[64,65] Karimzadeh and Farahpour[55] reported that *S. officinalis* played a significant role in promoting new vessel formation. Similarly, Güzel et al.[37] noted a marked increase in angiogenesis on the 7th and 14th days due to the effects of *S. kronenburgii* and *S. euphratica*. Irmak et al.[57] documented that *S. miltiorrhiza* amplified angiogenesis and tissue perfusion in burn wounds. In our research, while there was a notable rise in angiogenesis across all groups over time, distinct group differences emerged only on the 7th day of biopsy. Spesifically, the ST-2.5 group showed a significantly greater increase in angiogenesis compared to the control group. However, by the 14th day, no discernible differences were observed between the groups. Even though the ST-2.5 extract seemed to have certain advantages in promoting angiogenesis, its overall effectiveness in this regard, when evaluated across all time points, did not appear to be markedly significant.

Following a vascular injury, the inflammatory phase of wound healing, characterized as acute inflammation, becomes fully established. This phase can persist for up to 96 hours or potentially longer, especially if complications such as infections, trauma, or other adverse events arise. During this phase, neutrophils and macrophages work

to clear tissue debris resulting from the injury, primarily through phagocytosis and the release of degrading enzymes. While this cleanup process is essential for wound recovery, an overzealous inflammatory response can hamper the wound healing process.[66] The primary objective of acute inflammation is to stave off potential infections in the affected area.[38] Karimzadeh and Farahpour[55] found that *S. officinalis* notably decreased neutrophil infiltration and simultaneously elevated the count of mononuclear cells, particularly macrophages, in the initial stages. Güzel et al.[37] observed a decline in dermal inflammation with *S. kronenburgii* and *S. euphratica*, akin to the effects seen with Fitokrem. In our research, while the initial periods (3rd and 7th days) showed no significant group disparities regarding acute inflammation by the 14th day, both ST-2.5 and ST-5 groups exhibited a marked reduction compared to the control and vehicle groups. This observation might hint at the direct anti-inflammatory or possibly indirect antibacterial capabilities of the extracts. Supporting this, Tepe et al.[30] highlighted the antimicrobial and antioxidant properties of *S. tomentosa*. They found that while its aqueous extract lacked antimicrobial activity, water-insoluble extracts exhibited moderate antimicrobial action against a range of bacteria. Separate findings by Haznedaroglu et al.[17] identified antibacterial properties of *S. tomentosa*, albeit limited to *Escherichia coli, S. aureus,* and *Pseudomonas aeruginosa*. Other researchers, like Ulukanlı et al.[41] reported on the bactericidal and insecticidal attributes of *S. tomentosa* Miller's essential oil. Tan et al.[40] demonstrated the antimycobacterial and antifungal effects of four Salvia species (*S. tomentosa*, *S. cilicica*, *S. officinalis*, and *S. fruticosa*), employing both alcoholic extracts and essential oils in their analysis. Furthermore, Askun et al.[24,67] emphasized the antimicrobial efficacy of the essential oils of *S. fruticosa* and *S. tomentosa*. Beyond these established antimicrobial activities, *S. tomentosa* appears to have anti-inflammatory properties. This is underscored by the findings of Sawmiller et al.[68] who determined that the flavonoid lutein, derived from *S. tomentosa* led to a decrease in TNF-α and IL-1β levels in a traumatic brain injury model. Collectively, these studies affirm the antibacterial and anti-inflammatory roles of *S. tomentosa*, further corroborated by our observations on the 14th day for the *S. tomentosa* groups.

During the proliferation phase of wound healing, processes like angiogenesis, epithelialization, and fibroplasia take place. To regain a normal tissue structure, it's essential that the stromal elements within the extracellular matrix provide a foundational framework for healing. The presence of functional fibroblasts, myofibroblasts, endothelial cells, pericytes, and epithelial cells is vital. In secondary wound healing, fibrous connective tissue fills the void left by tissue loss.[66] Our research found that on the 7th day, the fibrosis level was higher in both the ST-2.5 and Fitokrem groups when compared to the vehicle group, although the difference not statistically significant. By the end of the 14th day, there was no discernible difference among the groups regarding fibrosis. Fibroblast maturation levels didn't show significant differences among groups on the 3rd and 14th days. However, on the 7th day, the vehicle group exhibited reduced maturation compared to other groups. These observations suggest that the vehicle might have temporarily hampered fibrosis and fibroblast maturation on the 7th day, but this effect seemed transient, as indicated by the 14th-day results. It aligns with the findings of Karimzadeh and Farahpour[55] who reported that *S. officinalis* positively impacts fibroblast proliferation during wound healing. Similarly, Güzel et al.[37] found no statistical variance in fibrosis scores on both the 7th and 14th days, which is consistent with our observations.

While the processes of hemostasis, inflammation, proliferation, and remodeling typically follow a sequential pattern during wound healing, the rate at which these phases manifest can vary across wounds. It's even possible to observe different healing phases within distinct regions of a single wound.[66] Our histopathological analyses suggest that *S. tomentosa* acts to suppress inflammatory cells in the advanced stages of wound healing, potentially promoting improved healing outcomes. The lack of significant differences in other histopathological parameters could be attributed either to the inherent variability in wound healing or the representativeness of the biopsy samples examined.

Conclusion

This research reveals that the 2.5% and 5% ointments of *S. tomentosa*, which, to the best of our knowledge, have not been previously examined in a wound model, may positively influence macroscopic wound closure. The therapeutic potential of *S. tomentosa* is likely attributed to its angiogenic, anti-inflammatory, and antioxidant properties. A significant limitation of our study is the inability to determine its specific components. However, future research can delve deeper by comparing the effects of various extraction methods of this plant on wound healing aligning these with content determination and more comprehensive molecular analyses.

Acknowledgement

This work was supported by Burdur Mehmet Akif Ersoy University, Scientific Research Project Office under Grant no. 0644-YL-20.

We thank to Prof Dr Osman TUGAY for the herbarium number, and Dr Ümit ERDOĞAN for the ointment preparation and Kale Naturel for the extraction process.

References

1. Kumar S, Wong P, Leaper D. What is new in wound healing? Turkish J Med Sci. 2004;34:147-160.
2. 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
3. Guo S, DiPietro LA. Factors Affecting Wound Healing. J Dent Res. 2010;89(3):219. doi:10.1177/0022034509359125
4. Pereira RF, Bártolo PJ. Traditional Therapies for Skin Wound Healing. Adv Wound Care. 2016;5(5):208. doi:10.1089/WOUND.2013.0506
5. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528-1542. doi:10.1177/147323000903700531
6. Amri B, Martino E, Vitulo F, et al. Marrubium vulgare l. leave extract: Phytochemical composition, antioxidant and wound healing properties. Molecules. 2017;22(11):1851. doi:10.3390/molecules22111851
7. Barku VY. Wound Healing: Contributions from Plant Secondary Metabolite Antioxidants. In: Wound Healing - Current Perspectives. IntechOpen; 2019. doi:10.5772/intechopen.81208
8. Bilir K, Tutun H, Sevin S, Kismali G, Yarsan E. Cytotoxic effects of Rhododendron ponticum L. extract on prostate carcinoma and adenocarcinoma cell line (DU145, PC3). Kafkas Univ Vet Fak Derg. 2018;24(3):451-457.
9. Chah K, Eze C, Emuelosi C, Esimone C. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. J Ethnopharmacol. 2006;104(1-2):164-167. doi:10.1016/J.JEP.2005.08.070
10. Rathore VS, Balachandar K. Three-dimensional finite element simulation of gas tungsten arc welding for β-titanium alloy. Proc Inst Mech Eng Part B J Eng Manuf. 2014;228(7):730-735. doi:10.1177/0954405413513248
11. Kareru PG, Keriko JM, Kenji GM, Thiong'o GT, Gachanja AN, Mukiira HN. Antimicrobial Activities of Skincare Preparations from Plant Extracts. African J Tradit Complement Altern Med. 2010;7(3):214. doi:10.4314/AJTCAM.V7I3.54777
12. Wagate CG, Mbaria JM, Gakuya DW, et al. Screening of some Kenyan medicinal plants for antibacterial activity. Phytother Res. 2010;24(1):150-153. doi:10.1002/PTR.2866
13. Gould SWJ, Fielder MD, Kelly AF, Naughton DP. Anti-microbial activities of pomegranate rind extracts: enhancement by cupric sulphate against clinical isolates of S. aureus, MRSA and PVL positive CA-MSSA. BMC Complement Altern Med. 2009;9. doi:10.1186/1472-6882-9-23
14. Yuniarti WM, Lukiswanto BS. Effects of herbal ointment containing the leaf extracts of Madeira vine (Anredera cordifolia (Ten.) Steenis) for burn wound healing process on albino rats. Vet World. 2017;10(7):808. doi:10.14202/VETWORLD.2017.808-813
15. Rhimi W, Hlel R, Salem I Ben, Boulila A, Rejeb A, Saidi M. Dittrichia viscosa L. Ethanolic Extract Based Ointment with Antiradical, Antioxidant, and Healing Wound Activities. Biomed Res Int. 2019;2019. doi:10.1155/2019/4081253
16. Fikru A, Makonnen E, Eguale T, Debella A, Abie Mekonnen G. Evaluation of *in vivo* wound healing activity of methanol extract of Achyranthes aspera L. J Ethnopharmacol. 2012;143(2):469-474. doi:10.1016/J.JEP.2012.06.049
17. Haznedaroglu MZ, Karabay NU, Zeybek U. Antibacterial activity of *Salvia Tomentosa* essential oil. Fitoterapia. 2001;72(7):829-831. doi:10.1016/S0367-326X(01)00335-5
18. Inngjerdingen K, Nergård CS, Diallo D, Mounkoro PP, Paulsen BS. An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. J Ethnopharmacol. 2004;92(2-3):233-244. doi:10.1016/j.jep.2004.02.021
19. Schmidt C, Fronza M, Goettert M, et al. Biological studies on Brazilian plants used in wound healing. J Ethnopharmacol. 2009;122(3):523-532. doi:10.1016/J.JEP.2009.01.022
20. Tümen G, Malyer H, Başer K, Öz Aydın S. Plants used in Anatolia for wound healing. In: Proceedings of the IVth International Congress of Ethnobotany. 2006:217-221.
21. Davis P. Flora of Turkey and the Aegean Islands. Edinburgh University Press; 1982.
22. Arslan M. Cultivation Potential of *Salvia Tomentosa* and S. aramiensis Under the Eastern Mediterranean Conditions. Sci Pap Ser A Agron. 2016;LIX:174-177.
23. Ulubelen A, Miski M, Neuman P, Mabry TJ. Flavonoids of *Salvia Tomentosa* (Labiatae). J Nat Prod. 1979;42(3):261-263. doi:10.1021/NP50003A002
24. Askun T, Tumen G, Satil F, Ates M. Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants. Pharm Biol. 2009;47(7):563-571. doi:10.1080/13880200902878069
25. Bayan Y, Genç N. Determination of Antifungal and Antioxidant Activities of *Salvia Tomentosa* Mill. Iğdır Üniversitesi Fen Bilim Enstitüsü Derg. 2017;7(4):17-

21.
26. Dinçer C, Tontul I, Çam IB, et al. Phenolic composition and antioxidant activity of *Salvia Tomentosa* Miller: Effects of cultivation, harvesting year, and storage. Turkish J Agric For. 2013;37(5):561-567. doi:10.3906/tar-1211-72
27. Estévez M, Ramírez R, Ventanas S, Cava R. Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. LWT - Food Sci Technol. 2007;40(1):58-65. doi:10.1016/j.lwt.2005.07.010
28. Comino-Sanz IM, López-Franco MD, Castro B, Pancorbo-Hidalgo PL. The Role of Antioxidants on Wound Healing: A Review of the Current Evidence. J Clin Med. 2021;10(16). doi:10.3390/JCM10163558
29. Salem FMA, Ibrahim HM. Dry fermented buffalo sausage with sage oil extract: Safety and quality. Grasas y Aceites. 2010;61(1):76-85. doi:10.3989/gya.075109
30. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia Tomentosa* Miller (Lamiaceae). Food Chem. 2005;90(3):333-340. doi:10.1016/j.foodchem.2003.09.013
31. Scrima M, Melito C, Merola F, et al. Evaluation of wound healing activity of salvia haenkei hydroalcoholic aerial part extract on *in vitro* and *in vivo* experimental models. Clin Cosmet Investig Dermatol. 2020;13:627-637. doi:10.2147/CCID.S224418
32. Pawar SS, Dasgupta D. Quantification of phenolic content from stem-bark and root of Hugonia mystax Linn. using RP-HPLC. J King Saud Univ - Sci. 2018;30(3):293-300. doi:10.1016/J.JKSUS.2016.09.002
33. Ben Mansour R, Ksouri WM, Cluzet S, Krisa S, Richard T, Ksouri R. Assessment of Antioxidant Activity and Neuroprotective Capacity on PC12 Cell Line of Frankenia thymifolia and Related Phenolic LC-MS/MS Identification. Evidence-based Complement Altern Med. 2016;2016. doi:10.1155/2016/2843463
34. Huang LP, Wang GQ, Jia ZS, Chen JW, Wang G, Wang XL. Paclitaxel reduces formation of hypertrophic scars in the rabbit ear model. Ther Clin Risk Manag. 2015;11:1089. doi:10.2147/TCRM.S82961
35. Ahmed RR, Mahmoud A, Ahmed OM, Metwalli A, Ebaid H. Up-regulation of Hsp72 and keratin16 mediates wound healing in streptozotocin diabetic rats. Biol Res. 2015;48(1):1-12. doi:10.1186/S40659-015-0044-5/TABLES/4
36. Gül Satar NY, Cangül IT, Topal A, Kurt H, Ipek V, Onel GI. The effects of Tarantula cubensis venom on open wound healing in rats. J Wound Care. 2017;26(2):66-71. doi:10.12968/jowc.2017.26.2.66
37. Güzel S, Özay Y, Kumaş M, et al. Wound healing properties, antimicrobial and antioxidant activities of Salvia kronenburgii Rech. f. and Salvia euphratica Montbret, Aucher & Rech. f. var. euphratica on excision and incision wound models in diabetic rats. Biomed Pharmacother. 2019;111(September 2018):1260-1276. doi:10.1016/j.biopha.2019.01.038
38. Broughton G, Janis JE, Attinger CE. The basic science of wound healing. Plast Reconstr Surg. 2006;117(7 SUPPL.). doi:10.1097/01.prs.0000225430.42531.c2
39. Budovsky A, Yarmolinsky L, Ben-Shabat S. Effect of medicinal plants on wound healing. Wound Repair Regen. 2015;23(2):171-183. doi:10.1111/wrr.12274
40. Tan N, Satana D, Sen B, et al. Antimycobacterial and antifungal activities of selected four Salvia species. Rec Nat Prod. 2016;10(5):593-603.
41. Ulukanli Z, Karabörklü S, Cenet M, Sagdic O, Ozturk I, Balcilar M. Essential oil composition, insecticidal and antibacterial activities of *Salvia Tomentosa* Miller. Med Chem Res. 2013;22(2):832-840. doi:10.1007/s00044-012-0075-1
42. Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines. Thompson PDR; 2000.
43. Tuladhar ET, Rao A. Plasma protein oxidation and total antioxidant power in premenstrual syndrome. Asian Pac J Trop Med. 2010;3(3):237-240. doi:10.1016/S1995-7645(10)60017-X
44. Wojdyło A, Oszmiański J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105(3):940-949. doi:10.1016/J.FOODCHEM.2007.04.038
45. Khorasani Esmaeili A, Mat Taha R, Mohajer S, Banisalam B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from *In vivo* and *In vitro* Grown Trifolium pratense L. (Red Clover). Biomed Res Int. 2015;2015. doi:10.1155/2015/643285
46. Erdogan-Orhan I, Baki E, Şenol S, Yilmaz G. Sage-called plant species sold in Turkey and their antioxidant activities. J Serbian Chem Soc. 2010;75(11):1491-1501. doi:10.2298/JSC100322115E
47. Firuzi O, Miri R, Asadollahi M, Eslami S, Jassbi AR. Cytotoxic, Antioxidant and Antimicrobial Activities and Phenolic Contents of Eleven Salvia Species from Iran. Iran J Pharm Res IJPR. 2013;12(4):801.
48. Agar O, Dikmen M, Ozturk N, Yilmaz M, Temel H, Turkmenoglu F. Comparative Studies on Phenolic Composition, Antioxidant, Wound Healing and Cytotoxic Activities of Selected Achillea L. Species Growing in Turkey. Molecules. 2015;20(10):17976-18000. doi:10.3390/MOLECULES201017976

49. Aker D, Nisbet C. Antioxidant activities, total phenolic and flavonoid contents of honey collected from different botanical origins. Ankara Üniversitesi Vet Fakültesi Derg. 2020;67(2):133-136. doi:10.33988/AUVFD.523745
50. Erturk Ö, Kalın S, Ayvaz MÇ. Physicochemical properties, bioactive components, antioxidant and antimicrobial potentials of some selected honeys from different provinces of Turkey. Br Food J. 2019;121(6):1298-1313. doi:10.1108/BFJ-04-2018-0261
51. Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacol Res. 2008;58(2):165-171. doi:10.1016/J.PHRS.2008.06.004
52. Yadav E, Singh D, Yadav P, Verma A. Antioxidant and anti-inflammatory properties of Prosopis cineraria based phenolic rich ointment in wound healing. Biomed Pharmacother. 2018;108:1572-1583. doi:10.1016/J.BIOPHA.2018.09.180
53. Jagetia GC, Rajanikant GK. Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound, in mice whole-body exposed to various doses of γ-radiation. J Surg Res. 2004;120(1):127-138. doi:10.1016/j.jss.2003.12.003
54. Guimarães I, Baptista-Silva S, Pintado M, Oliveira AL. Polyphenols: A Promising Avenue in Therapeutic Solutions for Wound Care. Appl Sci 2021, Vol 11, Page 1230. 2021;11(3):1230. doi:10.3390/APP11031230
55. Karimzadeh S, Farahpour MR. Topical application of Salvia officinalis hydroethanolic leaf extract improves wound healing process. Indian J Exp Biol. 2017;55(2):98-106.
56. Pintapagung T, Asawapattanakul T, Buasrithong N, Phunnanon M, Thaingkhan W. Effect of oral administration of chia (Salvia hispanica L.) seed extract on wound healing property in diabetic mice. Vet Integr Sci. 2020;18(3):153-171.
57. Irmak F, Kurt Yazar S, Şirvan SS, Serin M, Özağarı A, Karasoy Yeşilada A. Beneficial effects of Salvia miltiorrhiza in the healing of burn wounds: an experimental study in rats. J Plast Surg Hand Surg. 2018;52(4):229-233. doi:10.1080/2000656X.2018.1461631
58. Salimikia I, Aryanpour M, Bahramsoltani R, et al. Phytochemical and Wound Healing Effects of Methanolic Extract of Salvia Multicaulis Vahl. in Rat. J Med Plants Stud. 2016;15(57); 38-46.
59. Süntar I, Akkol EK, Enol FS, Keles H, Orhan IE. Investigating wound healing, tyrosinase inhibitory and antioxidant activities of the ethanol extracts of Salvia cryptantha and Salvia cyanescens using *in vivo* and *in vitro* experimental models. J Ethnopharmacol. 2011;135(1):71-77. doi:10.1016/j.jep.2011.02.022
60. Pastar I, Stojadinovic O, Yin NC, et al. Epithelialization in Wound Healing: A Comprehensive Review. Adv Wound Care. 2014;3(7):445-464. doi:10.1089/wound.2013.0473
61. Ben Amar M, Wu M. Re-epithelialization: advancing epithelium frontier during wound healing. J R Soc Interface. 2014;11(93):20131038. doi:10.1098/rsif.2013.1038
62. Duque GA, Descoteaux A. Macrophage cytokines: Involvement in immunity and infectious diseases. Front Immunol. 2014;5(OCT):491. doi:10.3389/fimmu.2014.00491
63. Farahpour MR, Mirzakhani N, Doostmohammadi J, Ebrahimzadeh M. Hydroethanolic Pistacia atlantica hulls extract improved wound healing process; evidence for mast cells infiltration, angiogenesis and RNA stability. Int J Surg. 2015;17:88-98. doi:10.1016/j.ijsu.2015.03.019
64. Harper D, Young A, McNaught CE. The physiology of wound healing. Surg (United Kingdom). 2014;32(9):445-450. doi:10.1016/j.mpsur.2014.06.010
65. Cerqueira MT, Pirraco RP, Martins AR, Santos TC, Reis RL, Marques AP. Cell sheet technology-driven re-epithelialization and neovascularization of skin wounds. Acta Biomater. 2014;10(7):3145-3155. doi:10.1016/j.actbio.2014.03.006
66. Ackermann MR. Inflammation and Healing. In: Zachary JF, ed. Pathologic Basis of Veterinary Disease. 6th ed. Elsevier; 2017:121-131.
67. Askun T, Hüsnü Can Başer K, Tümen G, Kürkçüoğlu M. Characterization of essential oils of some Salvia species and their antimycobacterial activities. Turkish J Biol. 2010;34(1):89-95. doi:10.3906/biy-0809-2
68. Sawmiller D, Li S, Shahaduzzaman M, et al. Luteolin reduces Alzheimer's disease pathologies induced by traumatic brain injury. Int J Mol Sci. 2014;15(1):895-904. doi:10.3390/ijms15010895