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Original Article

Assessment of the Impact of *Astragalus floccosus* Boiss. Extract on Wound Based on Phytoestrogenic Properties: An *In vitro* and *In vivo* Study

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What's Known

- *Astragalus floccosus* Boiss. has been utilized as a traditional medicinal remedy since ancient times to boost the immune system against infections, safeguard the liver, cure sex hormone-related diseases, and expedite the healing of wounds.

What's New

- This study evaluates the wound healing of *Astragalus floccosus* Boiss., a native Iranian plant. The extract enhances wound healing activity through cell migration and duplication *in vitro*, while *in vivo* it reduces necrosis, induces fibrosis, epithelization, and proliferation of fibroblasts. This effect may contribute to the high amount of flavonoids in the extract.

Abstract

Background: Investigations have shown that estrogen affects wound healing through skin receptors. *Astragalus floccosus* Boiss. contains estrogen-like isoflavonoids. This study examined the effects of *A. floccosus* Boiss (*A. floccosus*) extract on wound healing *in vitro* and *in vivo*.

Methods: For the *in vitro* test, Human Fetal Foreskin Fibroblast 2 (HFFF2) cells treated with the root extract of *A. floccosus* were followed over a 48-hour period. For the *in vivo* test, rats were categorized into five groups and received daily applications of a cream containing different concentrations of the extract: 5%, 10%, silver sulfadiazine, the ointment base, and no treatment for a duration of 21 days. The wound size and full-thickness wound healing were measured throughout the inspection, and a histological study was conducted as well. The two-way repeated measures ANOVA test was employed, with a significance level of 0.05 ($P \leq 0.05$) being used in all cases.

Results: *A. floccosus* at 125 ppm significantly improved the closer region and wound healing in HFFF2 cells over all periods of time and showed a significant healing effect during 8-12 h (P=0.0234). Moreover, an increase in fibrosis (compared to the base (P=0.0005), the 10% extract (P<0.0001), silver (P=0.0005), and free (P<0.0001) groups), epithelization (compared to base (P=0.0006), the 10% extract (P=0.0008), silver (P=0.0007), and free (P=0.0006) groups), and proliferation of fibroblasts (compared to the 10% extract and free groups (P=0.0020 and P=0.0024, respectively) were seen in the 5% extract sample on day 10.

Conclusion: Wound healing processes were observed in rats treated with a 5% *A. floccosus* extract cream. This finding highlights the potential for future studies on wound healing processes using this extract.

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Keywords ● *Astragalus* plant ● Wound healing ● Flavonoids ● Chromatography ● Cell line

Introduction

The wound healing process involves several types of cells, including keratinocytes, fibroblasts, endothelial cells, neutrophils, macrophages, and lymphocytes.[1] The wound

expresses inflammation through the synthesis of eicosanoids, prostaglandins, leukotrienes, and reactive oxygen species (ROS). The manifestation of this condition is characterized by edema, erythema, and discomfort. Regulating the body's inflammatory reactions will lead to expedited wound healing.[2] ROS play a crucial part in the healing process and function as cellular messengers. Additionally, ROS influence the cells surrounding the wound, which can disrupt the wound healing process.[3]

Despite recent improvements in wound healing pharmacological therapy, there is still a need to discover cost-effective medications, including readily available ingredients.

Astragalus floccosus Boiss *(A. floccosus)* has been utilized as a traditional medicinal remedy since ancient times to boost the immune system against infections, safeguard the liver, cure sex hormone-related malignancies, and expedite the healing of wounds and abscesses.[4] A recent study on flavonoids and isoflavonoids found that the *A. floccosus* genus revealed antioxidant, anti-inflammatory, and phytoestrogenic capabilities. The presence of these flavonoids synergistically contributes to the wound healing process by exhibiting both anti-oxidant and anti-inflammatory capabilities.[5] Flavonoids can scavenge radicals and regulate the immune response. Isoflavonoids, which possess structures such as estrogen, specifically attach to the estrogen receptor (ER) in humans and can induce the effects of estrogen activity in wound healing. Estrogens impact wounds by controlling a range of genes that are involved in the process of regeneration, extracellular matrix formation, protease inhibition, and functioning of the outermost layer of the skin. Genes are responsible for causing inflammation.[6] This estrogen related process of wound healing varies between young and elderly persons due to the fall in estrogen levels with age in both men and women.[7, 8]

Genistein, as an isoflavonoid, showed good estrogen-related effects in previous studies.[9-12] This study aimed to assess the contribution of *A. floccosus* extract in the process of wound healing in both *in vivo* and *in vitro* experiments.

Material and Methods

Materials

Rats and all materials such as silver sulfadiazine, cold cream, cell medium cultures, genistein, and methanol for extraction were provided by Mazandaran University of Medical Sciences. The North Research Pasteur Institute in Iran provided the human fetal foreskin cells of Caucasian origin (HFFF2) cell line.

Herbal Preparation

Plant Collection: *A. floccosus* was collected from its native habitat in Isfahan province, Iran, at a latitude of 32° 58´N and a longitude of 50° 01´E, during its flowering season on 5 June. The plant's authenticity was confirmed by Dr. Bagheri, an expert in systematic herbal research.[13, 14] Subsequently, herbarium samples were meticulously prepared and kept at the herbarium of the University of Isfahan (HUI) under the designated herbarium code 23019.

Plant Extraction: The process of maceration was employed to extract the desired components. For this purpose, 1 Kg of powdered root of *A. floccosus* was used. This involved using absolute methanol (4 L) at room temperature for 3 days. Subsequently, the extract was subjected to drying using a rotary evaporator (Heidolph, Germany) and freeze dryer (yield 100 g extract powder) (Zirbus, Germany), and then stored in a freezer at a temperature of -20 °C (The extraction efficiency was 10 %).

Analysis of the Genistein Level of Extract by High Performance Liquid Chromatography (HPLC): As mentioned in our earlier article, the flavonoid content in the root powder was 0.67 Kg/Kg, while the phenolic compound content was 0.00067 Kg/Kg using the spectrophotometry and aluminum chloride colorimetric techniques.[15] The presence of genistein was identified using the technique developed by Akbari and others.[16] A C18 column (Knauer, USA) of 53×7 mm was utilized. The gradient solvent system included a mixture of water-acetic acid (1 L of deionized water with 1.2 mL of acetic acid (Merk, Germany) and acetonitrile (Merk, Germany). The injection volume was 20 µL, and the acetonitrile solvent was gradually increased from 0 to 12% over 6 min, followed by a further increase from 23% to 100% over 15 min. The HPLC, equipped with a UV detector (Knauer, USA), was configured to have a solvent flow rate of 2.5 mL/min at a wavelength of 280 nm. The component genistein was used as an external standard (Merk, Germany). Additionally, the standard mixed with the extract (internal standard) was injected separately into the system.

In Vitro Assays

Evaluation of the In Vitro Wound-Healing Activity: The human fetal foreskin cells of Caucasian origin, known as the HFFF2 cell line, were acquired from the North Research Pasteur Institute of Iran. The cell culture was conducted using Oswell Park Memorial Institute Medium-1640 (RPMI-1640) (Biosera, Denmark)

culture media, which was supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 1% mixture of antibiotic/antimycotic (Gibco, USA), 0.01 M of 2-(4-[2-hydroxyethyl] piperazin-1-yl) ethanesulfonic acid (HEPES) (Gibco, USA), 0.02 M of L-glutamine (Gibco, USA), and 0.001 M of sodium pyruvate (Gibco, USA). The cells were then incubated in a humidified environment with 5% CO_2 at 37 °C in an incubator.[17] The scratch-wound test was employed to assess the wound-healing efficacy of the extract.[18]

HFFF2 cells were placed in 12-well plates at a density of 4×104 cells per well. The cells were then grown in RPMI media supplemented with additional nutrients until they formed a monolayer on each plate. Following the cells' attachment to the plates, the liquid medium was withdrawn, and a scratch was made in the center of the plates using the tip of the p200 micropipette. The plates were then rinsed with phosphate-buffered saline (PBS) to eliminate any cells that did not adhere and any remaining cellular debris.

After evaluating various doses of *A. floccosus* extract on the HFFF2 cell line, a dose of 125 ppm was selected. The solution was dissolved in RPMI-1640 and thereafter introduced into the wells. In the control group, cells were just treated with RPMI-1640 culture media supplemented with other substances. Subsequently, the sample was placed in an incubator set at a temperature of 37 °C and an atmosphere containing 5% carbon dioxide for 24 hours. The microscopic images were captured at intervals of 0, 2, 4, 6, 8, 12, and 24 hours using IC Measure software version 2.0.0.286 (The Imaging Source, Germany). The program was used to quantify the progress of the scratch closure region, which was then compared to the control group. The percentage reduction of the scratch is calculated as A1 formula: (T1 indicates the initial time and T2 indicates the final time of each evaluated episode of time)

A1: *Percent of reduction=(T2 Scratch Area-T1 Scratch Area)/T1 Scratch*

In Vivo Assessments

Preparation of the Herbal Ointment: To prepare the solution, 2.5 g of *A. floccosus* extract was combined with 2.5 mL of water. The extract was dissolved in the water, and 7.5 mg of Eucerin (Eucerin, Orvand, Iran) was added to it until the mixture was well blended. Subsequently, all the components were combined with 37.5 g of cold cream (cold cream, Orvand, Iran), increasing the total weight to 50 g. To create a 10% ointment of *A. floccosus* extract, 5 g of the extract was dissolved in 5 mL of water and then added to 15 mg of Eucerin. This mixture was then combined with 25 g of cold cream, resulting in a final weight of 50 g.

Animals: Male Wistar rats, weighing 200 to 220 g, were obtained from the Institute for Laboratory Animal Research of Mazandaran University of Medical Sciences. The ethical code IR.MAZUMS.REC.1400.330 obtained from the Research Ethics Committee of Mazandaran University of Medical Sciences, ensured the protection of animal rights, including the provision of standard conditions such as free access to food and water, a 12-hour light-dark cycle, and room temperature for the animals.

Wound Induction: A ketamine-xylazine (Xylamax, Rooyan Darou, Iran) (ketamine, Alfasan, Netherlands) combination was administered to anesthetize the rats via intraperitoneal injection, with a volume of 0.1 mL/100 g per body weight. The animal's dorsal hairs were removed, followed by the creation of a circular full-thickness incision measuring 1.5×1.5 cm on the backs of all animals.[19]

Animals and Experimental Design: 50 male rats were divided into five groups, with each group consisting of 10 rats. Each rat was housed in a separate cage. Groups 1-4 were subjected to daily topical treatment for a duration of 21 days. The treatment involved the application of 5% and 10% extract ointment in groups 1 and 2, respectively. Group 3 received silver sulfadiazine, which is a conventional drug, while group 4 was treated with an ointment base, namely cold cream. Group five did not receive any treatment as the control (free).

***Wound Size Evaluation*:** The dimensions of the wound were measured using IC measure software on days 1, 3, 5, 10, 14, and 21. The wound healing ratio was computed using the A2 formula:[20]

A2: *The ratio of wound healing=(Primary area-secondary area)/Primary area×100*

Tissue Collection: Rats were anesthetized on days 3, 5, 10, 14, and 21, and the skin tissues were collected. The specimens were immersed in a solution containing 10% formalin and then subjected to staining using Masson's trichrome stain (MT) and hematoxylin and eosin (H&E). The samples were analyzed for the presence of inflammation, necrosis, fibroblast growth, and epithelialization.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6 (Dotmatics, USA), and the findings were provided as mean±SD. The two-way repeated measures ANOVA test was employed, with a significance level of 0.05 ($P \leq 0.05$) used in all cases.

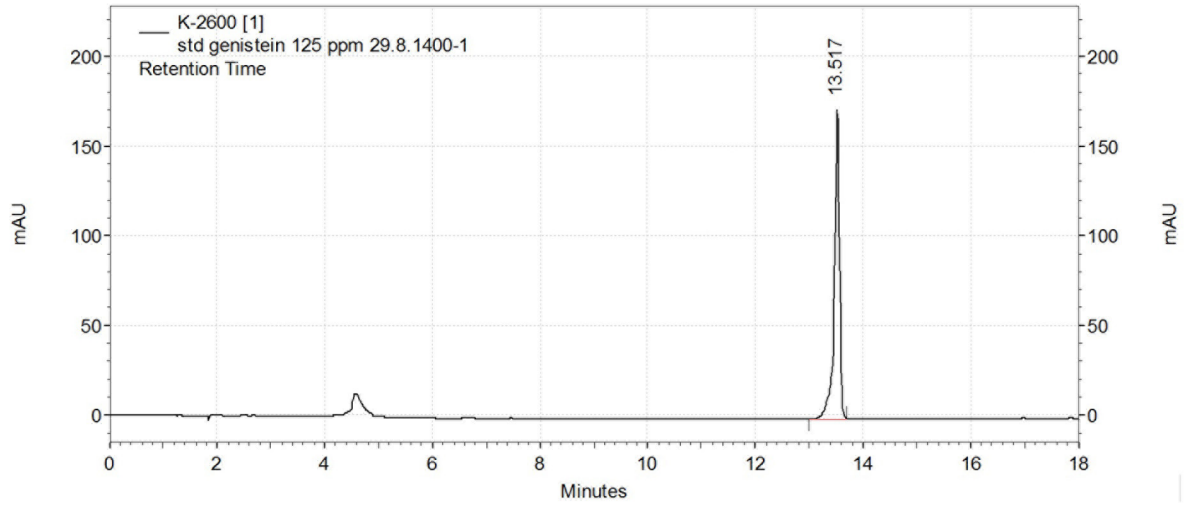

Figure 1: The 125-ppm standard genistein HPLC analysis shows the standard peak at a retention time of 13.517.

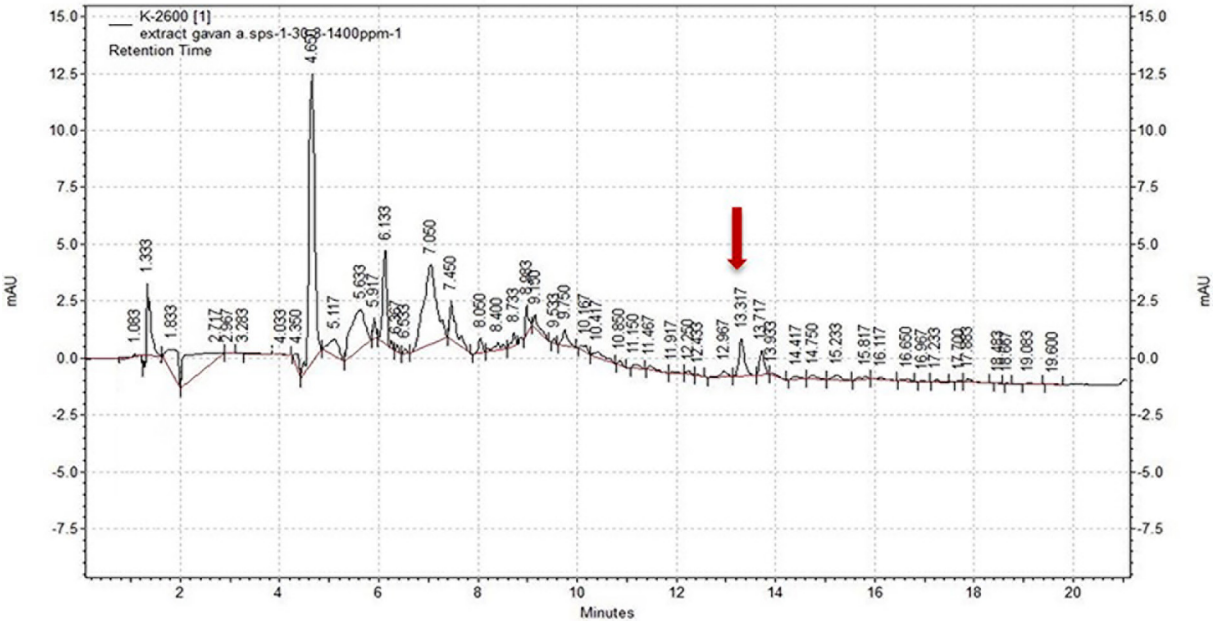

Figure 2: The HPLC diagram displays the extract at a concentration of 200 ppm. The red arrow indicates the location of the extract's genistein peak.

Results

HPLC Analysis of Genistein in the Extract

Figures 1, 2, and 3 display the diagrams of standard genistein, the extract, and the combination of the extract with the standard, respectively. Genistein was detected at retention times of 13.517, 13.317, and 11.342 min in the standard genistein diagrams, extract, and the standard-genistein mix, respectively. The extract group has a retention period of 13.317 min, as indicated by the red arrow. In addition, based on the standard curve of the standard genistein (Y=2751.9X +7414, R2=0.9905), the area under the extract curve at the time when the genistein peak appeared was trace (<0.0001% in the extract).

In Vitro Wound-Healing Activity

Throughout the investigation, HFFF2 cells were seen to proliferate and migrate in the group that received 125 ppm extract treatment. Neither group had any mortality. The extract group exhibited a better healing rate (percentage of growth) than the control group in all experimental time intervals, but a significant healing rate was seen in time episode 8-12 hours (P=0.0234). It was shown by the measurement of the scratch area (figure 4 and table 1).

The main effect of time for healing rate was F (2, 4)=9.691, P=0.0293. The main effect of group for fibrosis was F (1,2)=956.8, P=0.0010. The interaction effect of time×group was F(2,4)=2.843, P=0.1705. The effects of time and group were significant, but the interaction effect of time×group was not significant.

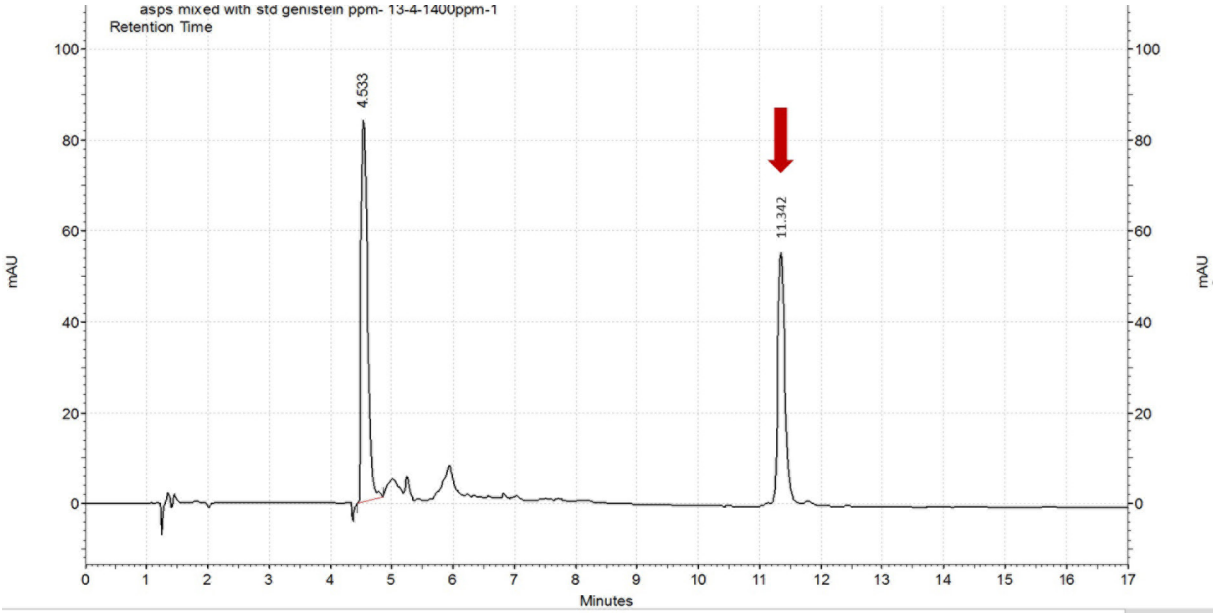

Figure 3: The HPLC diagram, combining the standard genistein (125 ppm) with the extract (200 ppm), displays a retention time of 11.342, which aligns with the genistein peak.

Table 1: The percentage of growth at time intervals of 0-2, 8-12, and 12-24 hours

Time interval	0-2 (h)		8-12 (h)		12-24 (h)	
Sample	**The percentage of growth**	**P value**	**The percentage of growth**	**P value**	**The percentage of growth**	**P value**
Control	25.34±3.54	0.0828	1.73±0.03	0.0234*	25.20±3.7	0.1818
Extract 125 ppm	43.35±1.75		52.09±3.9		39.62±4.5	

The percentage reduction of the scratch is calculated as (T2 Scratch Area-T1 Scratch Area) divided by T1 Scratch Area, which is approximately; *P<0.05 vs. control.

Table 2: Wound size in control (cold cream, non-treated), silver sulfadiazine (standard), and 5%/10% extract groups on days 3, 5, and 10

Sample	Day 3			Day 5			Day 10		
	P value Base#	**P value Free+**	**P value Silver^**	**P value Base#**	**P value Free+**	**P value Silver^**	**P value Base#**	**P value Free+**	**P value Silver^**
Base	-	>0.9999	>0.9999	-	0.0002 (+++)	>0.9999	-	>0.9999	>0.9999
Free	>0.999	-	>0.9999	0.0002 (###)	-	<0.0001 (^^^^)	>0.9999	-	>0.9999
Silver	>0.9999	>0.9999	-	>0.9999	<0.0001 (++++)	-	>0.9999	>0.9999	-
Extract 5%	>0.9999	>0.9999	0.957	0.0190 (#)	<0.0001 (++++)	>0.9999	0.2631	0.0032 (++)	0.0025 (^^)
Extract 10%	>0.9999	>0.9999	>0.9999	0.7901	<0.0001 (++++)	>0.9999	0.0653	0.0009 (+++)	0.0007 (^^^)

The two-way repeated measures ANOVA test was employed, with a significance level of 0.05 (P≤0.05) being used in all cases. #,+ and ^ refer to P values of each group to base, free, and silver, respectively.) (####P<0.0001 ###P<0.001 and ##P<0.01 vs. base), (++++P<0.0001 +++P<0.001 and ++P<0.01 vs. free), (^^^^P<0.0001 ^^^P<0.001 and ^^P<0.01 vs. silver).

Animal Wound Analysis

The wound underwent healing in nearly all groups by day 14, except the free group. The 5% extract group significantly expedited the wound healing process and exhibited superior results compared to the free group and silver group on days 5 (P<0.0001), and on day 10, it was also significant (P=0.0032, P=0.0025, respectively) (table 2 and figure 5). Furthermore, the 10% extract group showed better wound healing than the free group on day 5 (P<0.0001), and on day 10, it also exhibited better efficacy than the free and silver groups (P=0.0009, P=0.0007, respectively).

The main effect of time for inflammation was F(1,2)=31.62, P=0.0302. The main effect of group for inflammation was F(4,8)=13.53, P=0.0012.

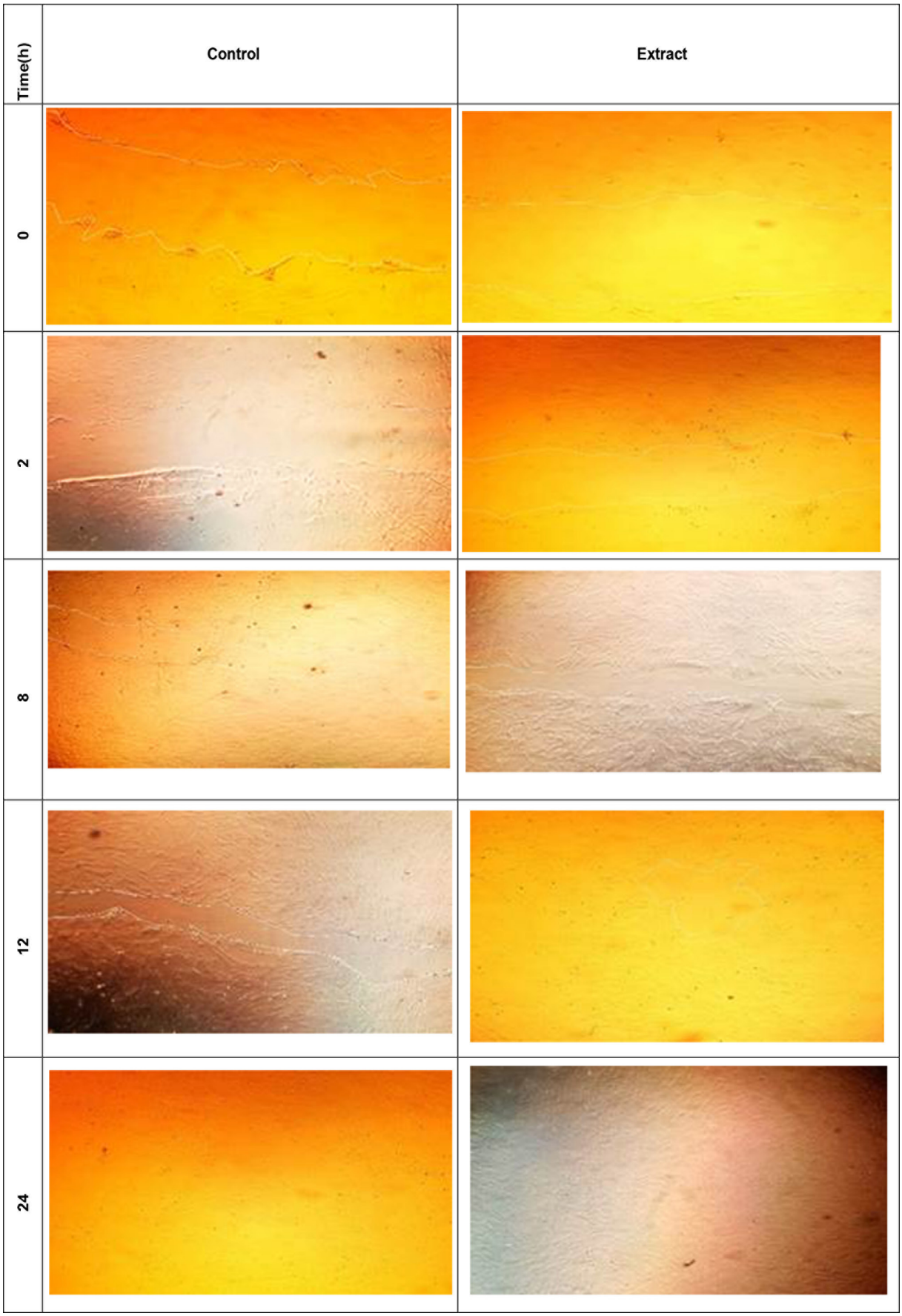

Figure 4: The microscopic images (100×) of scratch wound healing assay for (E) extract and (C) control samples. Images 1, 2, 3, 4, and 5 correspond to time intervals of 0, 2, 8, 12, and 24 hours after treatment, respectively.

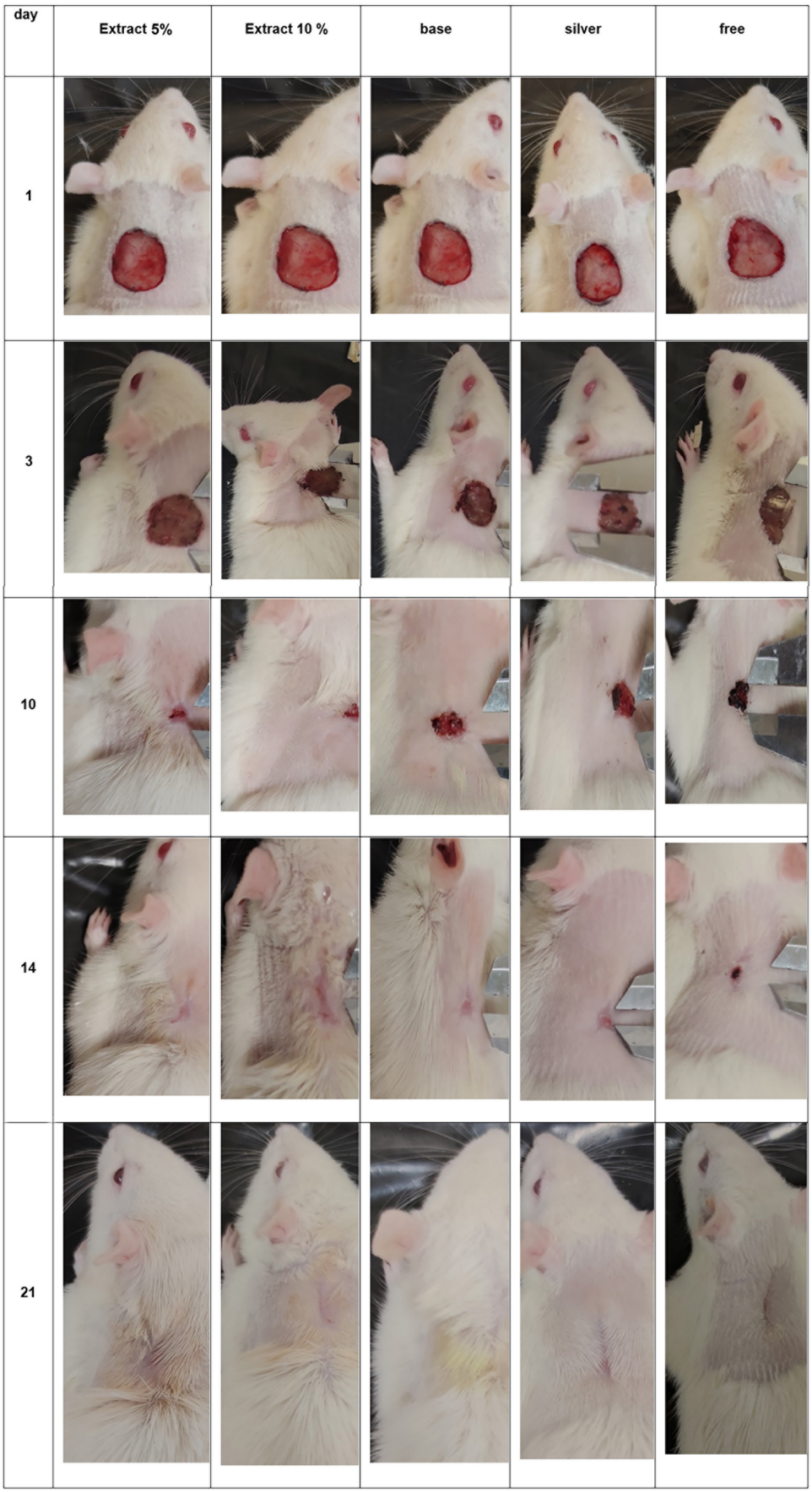

Figure 5: The wound that underwent a macroscopic evaluation on days 1, 3, 10, 14, and 21. All wounds in the experimental groups fully healed within a 14-day period, with the exception of the free group, which served as the non-treated control group.

The interaction effect of time×group was F(4,8)=6.73, P=0.0112. The effect of time, group, and the interaction effect of time× group were significant.

The main effect of time for necrosis was F(1, 2)=420.2, P=0.0024. The main effect of group for necrosis was F(4, 8)=24.95, P=0.0001. The interaction effect of time×group was F (4, 8)=5.641, P=0.0186. The effect of time, group, and the interaction effect of time×group were significant.

The main effect of time for the proliferation of fibroblasts was F(1,2)=21.56, P=0.0434. The main effect of group for the proliferation of fibroblast was F(4,8)=5.160, P=0.0236. The interaction effect of time×group was F(4,8)=24.03, P=0.0002. The effects of time, group, and the interaction effect of time×group were significant.

The main effect of time for fibrosis was F(1,2)=67.63, P=0.0145. The main effect of group for fibrosis was F(4,8)=6.186, P=0.0143. The interaction effect of time×group was F(4,8)=59.98, P<0.0001. The effects of time, group, and the interaction effect of time×group were significant.

The main effect of time for fibrosis was F(1,2)=181.1, P=0.0055. The main effect of group for fibrosis was F(4,8)=28.57, P<0.0001. The interaction effect of time×group was F(4,8)=36.09, P<0.0001. The effect of time, group, and the interaction effect of time× group were significant.

The main effect of time for wound size was F(1,2)=964.5, P<0.0001. The main effect of group for fibrosis was F(4,8)=59.21, P<0.0001. The interaction effect of time×group was F(4,8)=17.42, P<0.0001. The effect of time, group, and the interaction effect of time×group were significant.

Histopathological Investigation

The experimental procedure resulted in an increase in fibroblast proliferation and epithelization while reducing inflammation and necrosis. The extract 5% group has a significantly better impact on histopathological parameters such as epithelialization, fibroblast proliferation, inflammation, and fibrosis when compared to the silver sulfadiazine and free groups. The histopathological factors showed considerable similarity on days 3 and 5, but gradually became significantly different across the groups, particularly on days 10 and 14 (P<0.0001).

Fibroblast proliferation on day 10 was significantly higher in the 5% extract group than the 10% extract and free groups (P=0.0020 and P=0.0024, respectively).

The main effect of time for fibroblast proliferation was F(1,2)=21.56, P=0.0434. The main effect of group for fibrosis was F(4,8)=5.160, P=0.0236. The interaction effect of time × group was F(4,8)=24.03, P=0.0002. The effect of time, group, and the interaction effect of time× group were significant.

Moreover, the level of inflammation reduction in 5% extract was more than that of 10% extract (P=0.0396) on day 5. The main effect of time for inflammation was F(1,2)=31.62, P=0.0302. The main effect of group for fibrosis was F(4,8)=13.53, P=0.0012. The interaction effect of time ×group was F(4, 8)=6.731, P=0.0012. The effect of time, group, and the interaction effect of time×group were significant. The main effect of time for necrosis was F(1,2)=420.2, P=0.0024. The main effect of group for fibrosis was F(4,8)=24.95, P=0.0001. The interaction effect of time×group was F(4, 8)=5.641, P=0.0186. The effect of time, group, and the interaction effect of time×group were significant.

Moreover, epithelization in extract 5% was significantly better than base, extract 10%, silver, and free groups (P=0.0006, P=0.0008, P=0.0007, and P=0.0006, respectively) on day 10. On day 14, epithelialization in 5% and 10% extract groups was just better than in the free group (P≤0.0001). The main effect of time for epithelialization was F(1,2)=181.1, P=0.0055. The main effect of group for fibrosis was F(4,8)=28.57, P<0.0001. The interaction effect of time×group was F(4,8)=36.09, P<0.0001. The effect of time, group, and the interaction effect of time× group were significant.

On day 10, fibrosis was significantly better in the 5% extract group. This group showed significant differences compared to the base (P=0.0005), extract 10% (P<0.0001), silver (P=0.0005), and free groups (P<0.0001). Furthermore, on day 14, the 5% extract group showed significant differences compared to the free group (P=0.0019).

Moreover, on day 10, extract 10% fibrosis was significantly higher than that of the base and silver groups (P=0.0008 for both of them), and on day 14, the extract 10% showed a significant difference from that of the base and free groups (P=0.0017, P=0.0008, respectively). The main effect of time for fibrosis was F(1,2)=67.63, P=0.0145. The main effect of group for fibrosis was F(4,8)=6.186, P=0.0143. The interaction effect of time×group was F(4,8)=59.98, P<0.0001. The effect of time, group, and the interaction effect of time× group were significant.

The blue zones in Masson's trichrome dye samples indicated the tissue proliferation of fibroblasts, which can be attributed to collagen bond formation (figure 6).

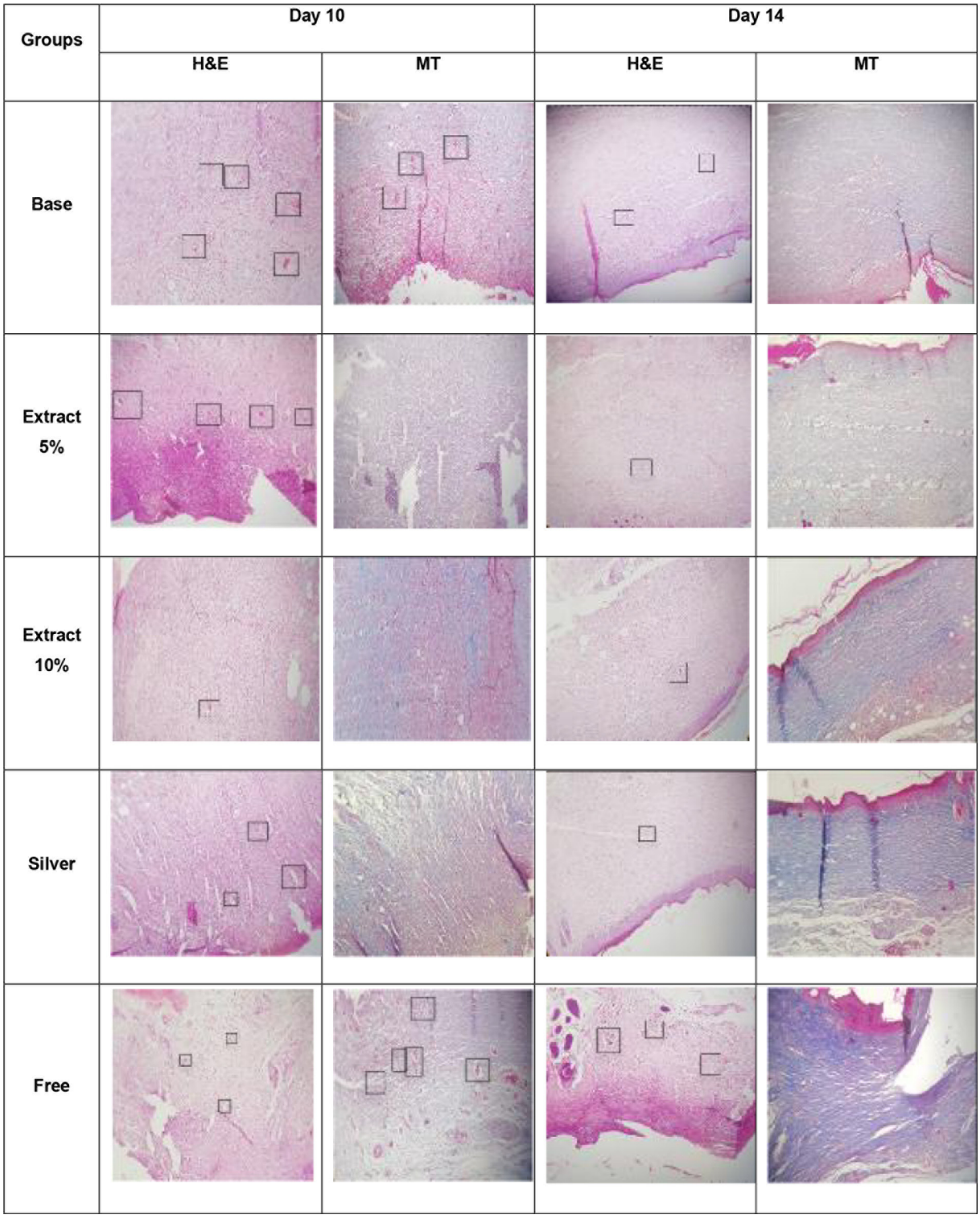

Figure 6: The microscopic images (40×) of samples obtained from base (cold cream), free (non-treated), silver (silver sulfadiazine), and extracts of 5% and 10%. On days 10 and 14. Samples were stained with MT and H&E on days 7 and 14. Squares represent changes in morphology, including inflammation, necrosis, and angiogenesis.

Discussion

The current investigation showed that *A. floccosus* extract improved the wound healing process in both *in vitro* and *in vivo* tests.

The extract-treated group exhibited considerably higher rates of cell migration and closure of the scratch compared to the control group in all time intervals (excluding episodes 4-6) following treatment in HFFF2 cells. This indicates that the extract effectively improved the pace of wound healing.

Afonso researched to assess the wound-healing effects of propolis and honey in a laboratory setting using a scratching test on Normal Human Dermal Fibroblasts (NHDF) cell lines.[21] In our prior investigation, we assessed the efficacy of this extract in promoting wound healing on the NHDF cell line.[22] Although the tolerance of this cell line extends to 500 ppm, the HFFF2 cell, in line with the investigation, showed tolerance to an extract of 125 ppm and exhibited a response to this dosage.

Numerous studies assessed wound-healing efficacy in animal models.[23] The predominant animals used for this purpose were rats, with several studies employing groups of 10 animals.[24-26] This investigation divided 50 rats into five groups, each containing 10 rats.

The current study demonstrates that *A. floccosus* ointments promote collagen production in wound tissue, thereby considerably improving the wound healing process. This result aligns with the results reported by Qian and others.[27] In a previous study, we utilized vaseline as the base of ointment for topical administration on the skin.[22] In our previous study, we noted a zero kinetic extract absorbance in the vaseline-based mixture.[15] In this investigation, cold cream was utilized as an inert base ointment instead of vaseline. Vaseline, although often employed in therapy, is not pharmacologically inert and has a notable impact on wounds, particularly burn wounds. Additionally, while our previous study focused on examining a 1.5% extract, the present study involved investigating two different concentrations of 5% and 10% extracts to further investigate their effects.[15] The histological analysis revealed that the groups treated with the extract exhibited increased fibroblast proliferation and epithelialization compared to the control groups. Additionally, there was a significant decrease in inflammation in the extract groups compared to the other groups. On day 10, the group treated with a 5% extract showed considerably greater improvement than the group treated with a 10% extract. However, this difference in effectiveness was not seen on day 14. Moreover, the present investigation identified fibrosis and collagen bonds by the utilization of Masson's trichrome stain, which revealed blue zones. The study observed that the extract groups exhibited a modulation of the body's inflammatory responses, leading to wound healing.

The three stages of wound healing that replace the normal tissue in the wounded area are remodeling, proliferation, and inflammation. Inflammation is an initial physiological response to wounds, triggering the activation of immune cells and affecting dermal cells to remove dead and damaged cells, subsequently replacing them with new, functioning cells.[3] It can stimulate an increase in cell death and aging with ongoing oxidative stress, lipid peroxidation, protein alteration, and DNA damage.[28] Insufficient levels of estrogen diminish the body's ability to protect against oxidative stress. Phytoestrogens bind to estrogen receptors (ER) and stimulate the formation of hyaluronic acid, collagen, and vascularization in the skin. They also enhance cell proliferation and protect cells from oxidative damage.[29] Genistein exhibits a structural similarity to estrogen, allowing it to indirectly safeguard against reactive oxygen species (ROS). It achieves this by inhibiting the activation of Mitogen-activated protein kinase (MAPK), suppressing the stimulation of the Nuclear factor kappa B (NF-kB) signaling pathway, and regulating the production of genes associated with antioxidants, such as glutathione peroxidase and manganese superoxide dismutase. Genistein has been employed as a selective inhibitor of tyrosine kinase. In addition, genistein stimulates the cyclic adenosine monophosphate (cAMP) signaling pathway by augmenting Insulin-like growth factor 1 (IGF-1) signaling and promoting an increase in IGF-1 receptor expression.[30] The presence of ERs α and β in the skin indicates that the extract compounds have an affinity for these receptors. This affinity could potentially explain the extract's wound-healing effect. This finding aligns with Özay and colleagues' research, which demonstrated that Kaempferol has wound-healing properties in both diabetic and non-diabetic models.[31]

The study's limitations included evaluating the amount of each extract's secondary metabolites and its effect on wound healing. It is recommended to assess the impact of estrogen on receptor function under both *in vitro* and *in vivo* conditions.

Conclusion

A. floccosus Boiss. demonstrated acceptable wound-healing activity *in vivo* (5% extract) and *in vitro* (125 ppm). The presence of flavonoids and their anti-inflammatory, anti-oxidant, and estrogenic effects contributes to this effect. This finding highlights the potential for further studies on wound healing processes using *A. floccosus* extract.

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Authors' Contribution

F.A: Conceptualization, Formal analysis, Methodology, Visualization, and Writing original draft; S.J.H: Data curation, Methodology, Project administration, and Validation; M.N: Data curation, Formal analysis, Validation, and Writing original draft; F.E: Data curation, Formal analysis, Methodology, Validation, and Writing original draft; A.B: Data curation, Resources, Validation, and Writing original draft; L.V: Data curation, and M.A: Conceptualization, Data curation, Methodology, Project administration, Supervision, and Validation. All authors have reviewed and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of Interest: None declared.

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