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Original Research Article

Effect of hydroalcoholic extracts of *Quercus brantii* and *Salvia officinalis* on the expression of wound healing and anti-inflammatory factors in human fibroblast cells

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Abstract

Objective: Based on research regarding the regenerative properties of *Quercus brantii* in wound healing and the anti-inflammatory benefits of *Salvia officinalis* in promoting the wound healing process, this study aims to explore the wound healing and anti-inflammatory effects of hydroalcoholic extracts of *Q. brantii* and *S. officinalis*, alone or in combination, on human fibroblast cells.

Materials and Methods: Hydroalcoholic extracts of *Q. brantii* and *S. officinalis* were prepared. Human fibroblast cell lines (HDF-1 cells) were treated with 1 to 256 µg/ml *Q. brantii* and/or *S. officinalis* extracts for 48 and 72 hr. Viability and migration potential of HDF-1 cells were evaluated utilizing MTT colorimetric and wound healing methods, respectively. Additionally, IL-6 (Interleukin-6), TNF-α (Tomur Necrose Factor-α), and VEGF (Vascular endothelial growth factor) levels were determined using ELISA kits and the expression of *collagen I* and *III* genes was estimated using real time PCR.

Results: After 48 and 72 hr, *Q. brantii* extract (8 µg/ml) and/or *S. officinalis* extract (16 µg/ml) significantly increased cell proliferation and decreased the migration ability of HDF-1 cells ($p \leq 0.05$). Treatment with an optimum concentration of *Q. brantii* and *S. officinalis* extracts significantly decreased the TNF-α level in HDF-1 cells ($p \leq 0.05$). The individual extracts of *Q. brantii* and *S. officinalis* did not produce a statistically significant effect on the concentration of IL-6 protein. However, the combination of these extracts resulted in a significant reduction in IL-6 levels ($p \leq 0.05$). Both extracts also caused a significant decrease in VEGF concentration ($p \leq 0.05$) and significantly increased the expression of *collagen I* and *III* genes ($p \leq 0.05$).

Conclusion: The combination *of Q. brantii* and *S. officinalis* extracts can accelerate the wound healing process by promoting fibroblast cell proliferation, reducing inflammation, and enhancing *collagen I* and *III* gene expression.

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Introduction

The skin, the body's largest organ, serves as the primary defense against injury and it is vital for maintaining homeostasis. Wounds disrupt the skin's structure and function, significantly affecting healthcare costs (1). Fibroblast cells are considered the most important cells in wound tissue repair. These cells participate in the wound healing process by penetrating and destroying the fibrin clot by secreting various metalloproteinases, creating a replacement for extracellular matrix by secreting substances such as collagen, proteoglycans, laminin, thrombus formation, glycosaminoglycans, and hyaluronic acid and contraction of open wound surface (2). Wound healing is facilitated by the action of cytokines, chemokines, and growth factors that are synthesized by fibroblasts, keratinocytes, and inflammatory cells that migrate to the site of injury (3).

The recent focus has been on developing new plant-based drugs to minimize the side effects of chemical drugs applied in wound healing (4); (5).

Quercus brantii is a medicinal plant of the family Fagaceae that grows in the forests of Iran (6). *Q. brantii* hydroalcoholic extract is effective in the proliferation and migration of 3T3 mouse fibroblastic cells, which are considered the main determinants of wound closure rate (7). On the other hand, *Salvia. officinalis* is the largest genus of this family. An ointment prepared from *S. officinalis* oil significantly increases pro-inflammatory cytokines and reduces the total number of bacteria in the wound. The expression levels of *IL-6* (Interleukin-6), *IL-1 β* (Interleukin-1β), and *TNF-α*(Tumor Necrose Factor-α) genes are increased by topical *S. officinalis* treatment, which means that this oil exacerbates the pro-inflammatory response in fibroblast cells (8).

Building on previous research into the regenerative effects of *Q. brantii* on wound healing and the anti-inflammatory properties of *S. officinalis* on fibroblast cell growth, invasion, and proliferation, this study aimed to investigate the effects of hydroalcoholic extracts of these medicinal plants, alone and in combination, on the proliferation and growth of human fibroblast cells. Additionally, we examined their effects on the expression of key wound healing factors (*collagen genes I* and *III*), inflammatory markers (IL-6 and TNF-α), and VEGF.

Materials and Methods

Plant materials and extract preparation

In early spring 2024, fruits of *Q. brantii* and aerial parts of S. *officinalis* were collected from various sites within Khuzestan Province, located in the southwestern region of Iran, and subsequently underwent purification to eliminate impurities. Following validation by a botanist, the plant materials were thoroughly washed, air-dried, and ground into a powder. The extraction process involved the use of 70% ethanol, with a ratio of 1 g of plant material to 10 ml of ethanol, and was conducted over a period of 72 hr at a temperature of 37°C, with intermittent shaking. The resulting mixture was then filtered using Whatman No. 1 filter paper and allowed to evaporate at room temperature. Ultimately, the extracts were reconstituted in a serum-free culture medium and sterilized using 0.22 µm syringe filters (9).

Cell culture

Human fibroblast cell line (HDF-1) (Pasteur Institute, Iran) was cultured in a monolayer in DMEM/F12 cell culture medium, supplemented with 10% FBS (Fetal Bovine Serum) and antibiotics (penicillin/streptomycin; 100 U/ml). The cells were maintained in a 37°C humidified incubator equipped with 5% CO_2.

Cell proliferation assay

HDF-1 cells were cultured in 96-well plates at a density of 15,000 cells per well and allowed to incubate overnight.

Following cell attachment, the cultures were subjected to treatment with varying concentrations of *Q. brantii* and *S. officinalis* extracts, specifically at 1, 2, 4, 8, 16, 32, 64, 128, and 256 µg/ml. After 48 and 72 hr of treatment, 50 µl of MTT solution (Sigma, Germany) at a concentration of 5 mg/ml was introduced to each well and incubated in the dark. After a 4-hr incubation period, 100 µl of Dimethyl sulfoxide (DMSO) (Sigma, Germany) was added to each well, and the plate was subsequently placed on a shaker at room temperature for 20 min. The absorbance was measured at a wavelength of 570 nm, with a reference wavelength of 630 nm, using an ELISA reader (BioTek, USA). The percentage of cell viability was determined by calculating the ratio of the absorbance of the treated group to that of the control group, multiplied by 100 (9).

Only the combination of *Q. brantii* and *S. officinalis* extracts at 8 and 16 µg/ml significantly increased cell proliferation.

Cell migration assay

At a density of 5×10^5 cells per well, cells were cultured in 6-well plates. Subsequently, a wound was induced in the cell monolayer using a micropipette tip with a volume range of 10–100 µl. Following this, the cells were rinsed with phosphate-buffered saline (PBS) and treated with extracts of *Q. brantii* and *S. officinalis*, both individually and in combination. After incubation periods of 48 and 72 hr, the wells were examined and photographed using a light microscope. The resulting images were subsequently analyzed utilizing Tathcratch software (MathWorks Inc, USA) (9).

ELISA

After treatment for 48 and 72 hr, cell culture supernatant was centrifuged at 10,000 g at 4°C for 20 min and collected. The concentration of IL-6 and TNF-α in cell culture supernatant was determined using ELISA kits (Proteintech, USA) according to the manufacturer's recommendations. An ELISA reader (BioTek, USA) was applied to measure the absorbance at 450 nm.

Real time PCR

The individual and combined effects of the extracts on the expression levels of *collagen I and III* genes were assessed through Real-Time Polymerase Chain Reaction (PCR). The primers utilized in this study (as detailed in Table 1) were designed using Gene Runner software, verified via NCBI Primer Blast, and procured from Pishgam Company located in Tehran, Iran. Total RNA was extracted employing a total RNA isolation kit from DENA Zist, also based in Tehran, Iran. The synthesis of complementary DNA (cDNA) was conducted using a cDNA synthesis kit from Vivantis Technologies, Selangor DE, Malaysia. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilized as an internal control, and the relative expression levels of each target mRNA were quantified using the comparative Ct (2-ΔΔct) method. Real-Time PCR was executed utilizing SYBR Premix Ex Taq Technology from Takara Bio Inc., Shiga, Japan, on the Applied Biosystems Step One Real-Time PCR System (refer to Table 1 for details).

Table 1. The primer sequences of genes.

No.	Gene	Sequence	Accession
1	*GAPDH*	F-5-CTTTGGTATCGTGGAAGGAC-3 R-5-GCAGGGATGATGATGTTCTGG-3	M_054337760
2	*collagen -I*	F-5-TGGAGCAAGAGGCGAGAG-3 R-5-CACCAGCATCACCCTTTAGC-3	XM_011533768
3	*collagen* -III	F-5-CTCCTACTCGCCCTCCTAATG-3 R-5-GAGGACCAGTAGGGCATGATT-3	NM_000090

Statistical analysis

All experiments were independently replicated a minimum of three times. Data are presented as means ± standard deviation (SD). Group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was defined as $p<0.05$.

Results

The effect of *Q. brantii* fruits and/or *S. officinalis* hydroalcoholic extracts on proliferation of fibroblast cells

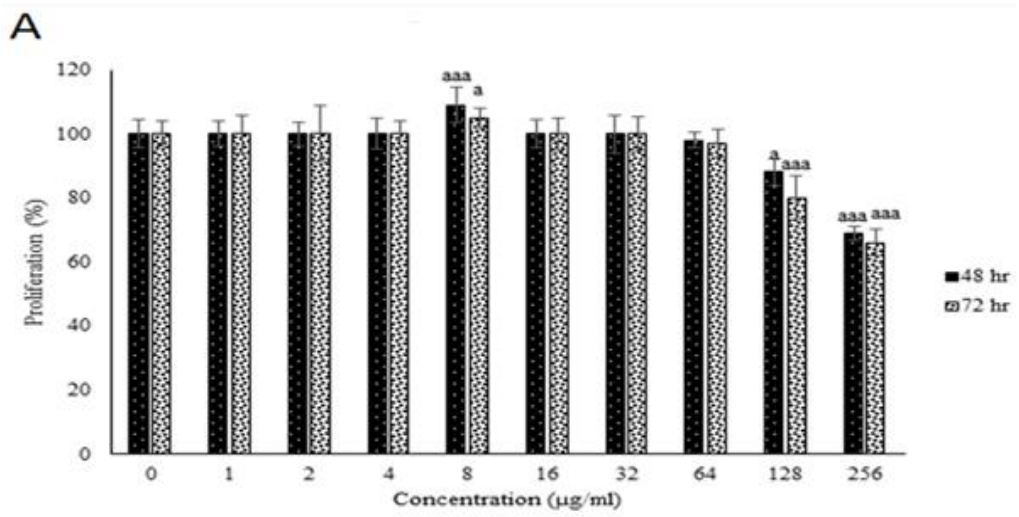
After 48 and 72 hr, 8 µg/ml concentration of *Q. brantii* fruit extract, caused a significant increase of 1.09 and 1.05 times in cell proliferation compared to the control group ($p\leq0.05$). This extract significantly reduced cell survival at concentrations of 128 and 256 µg/ml compared to the control group ($p\leq0.05$). Also, after 48 and 72 hr, 16 µg/ml concentration of *S. officinalis* hydroalcoholic extracts, caused a significant increase in cell proliferation by 1.06 and 1.06 times compared to the control group ($p\leq 0.05$). This extract had no toxicity in the concentrations used compared to the control group.

The combined treatment with *Q. brantii* (8 µg/ml) and *S. officinalis* (16 µg/ml) extracts over 48 and 72 hr significantly enhanced fibroblast cell proliferation compared to individual treatments ($p\leq0.05$) (Figure 1).

The effect of *Q. brantii* fruits and/or *S. officinalis* hydroalcoholic extracts on migration potential of fibroblast cells

After 48 hr, 16.01%, 18.76%, 25.15%, and 50.94% scratch closure was seen in the control group, *Q. brantii* fruits extract group, and *S. officinalis* extract, respectively. The changes in all groups were significant compared to the scratch created at hr 0 in the control group ($p\leq0.05$).

The results showed that after 72 hr, 17.55%, 22.83%, 28.37%, and 68.94% scratch closure was seen in the control group, *Q. brantii* fruits extract optimum concentrations group, and *S. officinalis* extract optimum concentrations group, respectively. The changes in all groups were significant compared to the scratch created at hr 0 in the control group ($p\leq0.05$) (Figure 2).



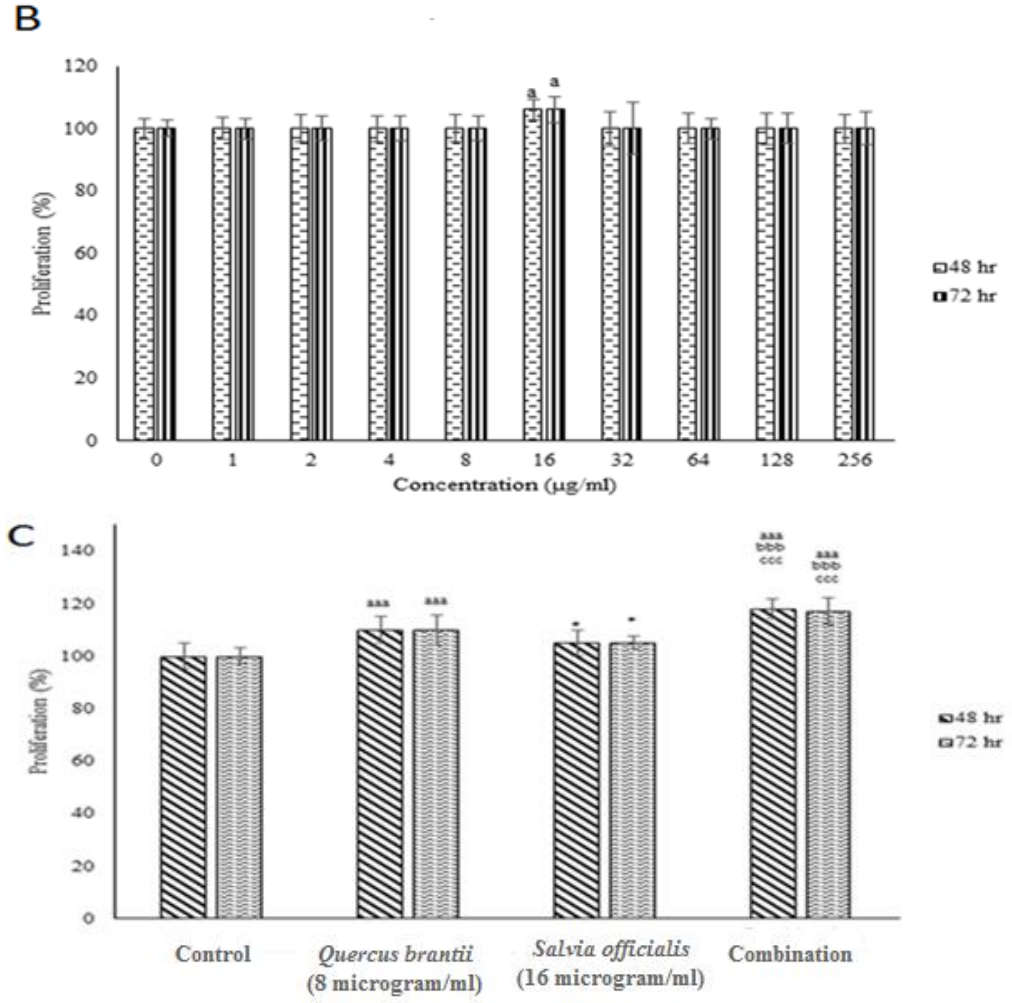


Figure 1. The effect of *Quercus brantii* extract (A), *Salvia officinalis* extract (B) and the combination of the two extracts (C) on the proliferation and survival of fibroblast cells. Control cells received the same volume of culture medium without extract. [a] indicates p≤0.05 and [aaa] indicates p≤0.001 compared to the control group, [bbb] indicates p≤0.001 compared to *Quercus brantii* extract group and [ccc] indicates p≤0.001 compared to *Salvia officinalis* extract group. All results are expressed as mean and SE obtained from three independent replicates for each treatment.

The effect of *Q. brantii* fruits and/or *S. officinalis* hydroalcoholic extracts on inflammatory factors levels in fibroblast cells

48 and 72 hr treatment with *Q. brantii* fruits and *S. officinalis* extracts at optimum concentrations led to a significant decrease in TNF-α level in the cells compared with the control group (p≤0.05) and the use of both extracts at the same time had a more significant reduction effect than each extract alone (p≤ 0.05). Also, *Q. brantii* fruits and *S. officinalis* extracts alone did not have a significant effect on IL-6 level compared to the control group (p≥0.05), but the combination of both extracts significantly decreased IL-6 level in fibroblast cells compared to the control group (p≤0.05) (Figure 3).

The effect of *Q. brantii* fruits and/or *S. officinalis* hydroalcoholic extracts on VEGF level in fibroblast cells

Q. brantii fruits and/or *S. officinalis* hydroalcoholic extracts after 48 and 72 hr, caused a significant decrease in VEGF level in the cells compared to the control group (p≤0.05) and the use of both extracts at the same time had a more significant reduction effect than each extract alone (p≤0.05) (Figure 3).

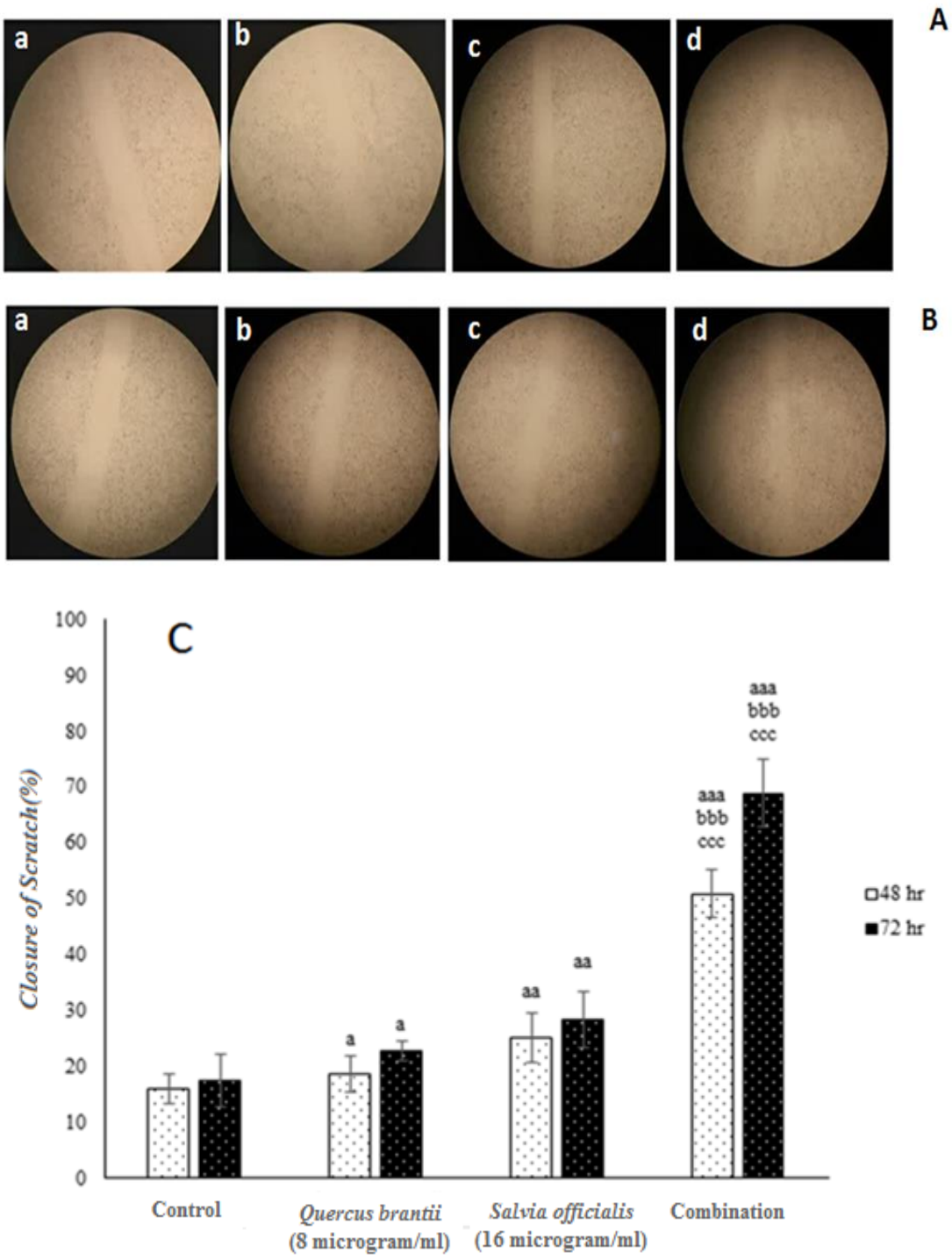


Figure 2. The effect of 8 µg/ml of *Quercus brantii* extract (b), 16 µg/ml of *Salvia officinalis* extract (c), and the combination of the two extracts (d) for 48 hr (A) and 72 hr (B) on the migration ability of fibroblast cells. Control cells (a) received the same volume of culture medium without extract. The images were taken using the X4 lens. A bar chart is shown in part I. ^{a} indicates $p\leq0.05$, ^{aa} $p\leq0.01$, and ^{aaa} $p\leq0.001$ compared to the control group, ^{bbb} indicates $p\leq0.001$ compared to *Quercus brantii* extract group, and ^{ccc} indicates $p\leq0.001$ compared to *Salvia officinalis* extract group. All results are expressed as mean and SE obtained from three independent replicates for each treatment (magnification, × 10).

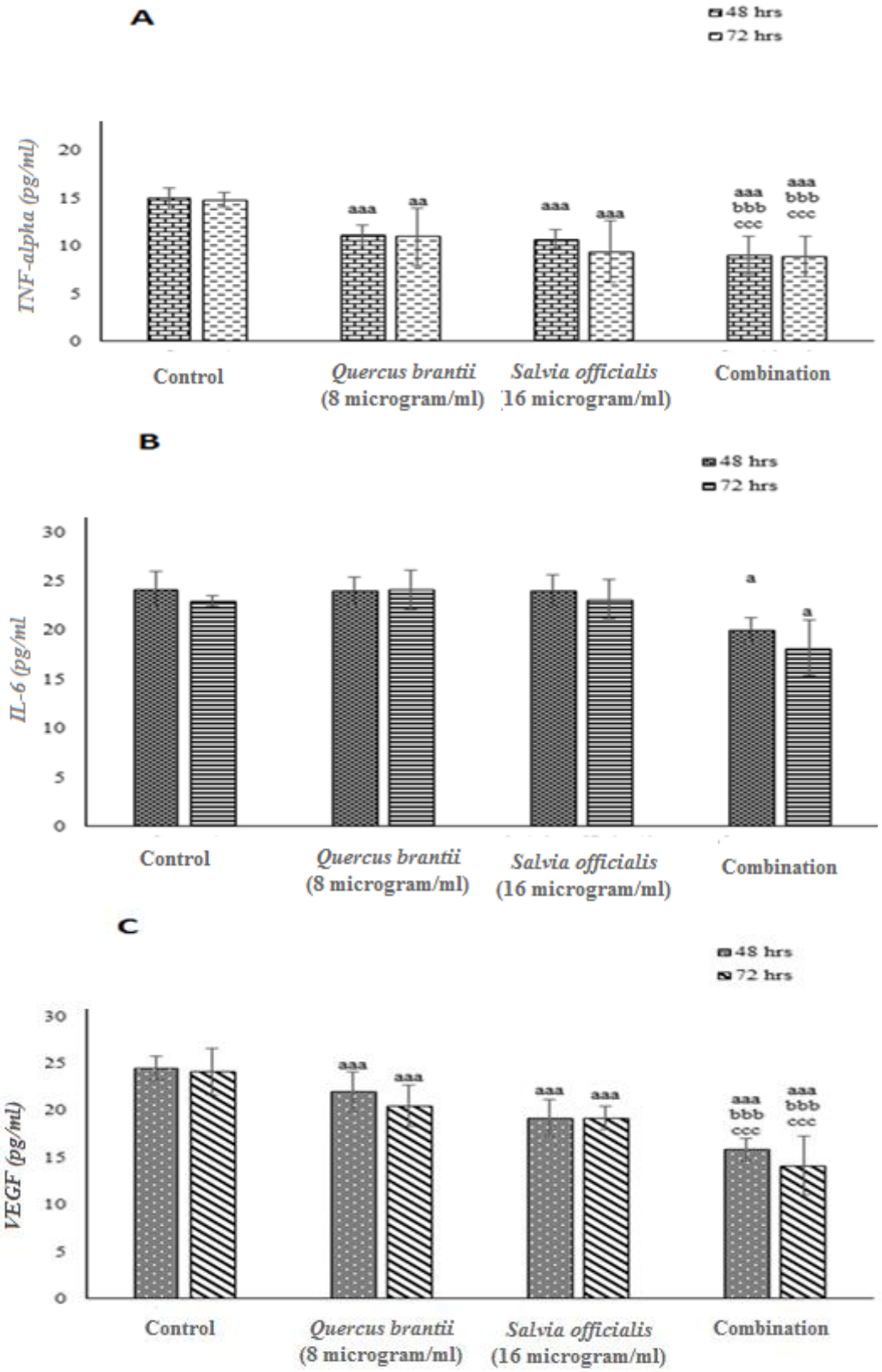


Figure 3. The effect of 8 and 16 µg/ml of *Quercus brantii* and *Salvia officinalis* extracts, respectively, and the combination of two extracts the TNF-α, IL-6, and VEGF levels in fibroblast cells after 48 and 72 hr of treatment. Control cells received the same volume of culture medium without extract. [a] indicates $p \leq 0.05$, [aa] indicates $p \leq 0.01$, and [aaa] indicates $p \leq 0.001$ compared to the control group, [bbb] indicates $p \leq 0.001$ compared to *Quercus brantii* extract group, and [ccc] indicates $p \leq 0.001$ compared to *Salvia officinalis* extract group. All results are expressed as mean and SE obtained from three independent replicates for each treatment.

The effect of *Q. brantii* fruits and/or *S. officinalis* hydroalcoholic extracts on *collagen I* and *III* genes expression in fibroblast cells

Q. brantii fruits and/or *S. officinalis* hydroalcoholic extracts after 48 and 72 hr significantly increased *collagen I* and *III* gene expression in cells compared to the control group ($p \leq 0.05$) and the use of combination treatment had a more increasing effect compared to each extract alone ($p \leq 0.05$) (Figure 4).

The results showed that after 72 hr, 17.55%, 22.83%, 28.37%, and 68.94% scratch closure was seen in the control group, *Q. brantii* fruits extract optimum concentrations group, and *S. officinalis* extract optimum concentrations group, respectively. The changes in all groups were significant compared to the scratch created at hr 0 in the control group ($p \leq 0.05$).

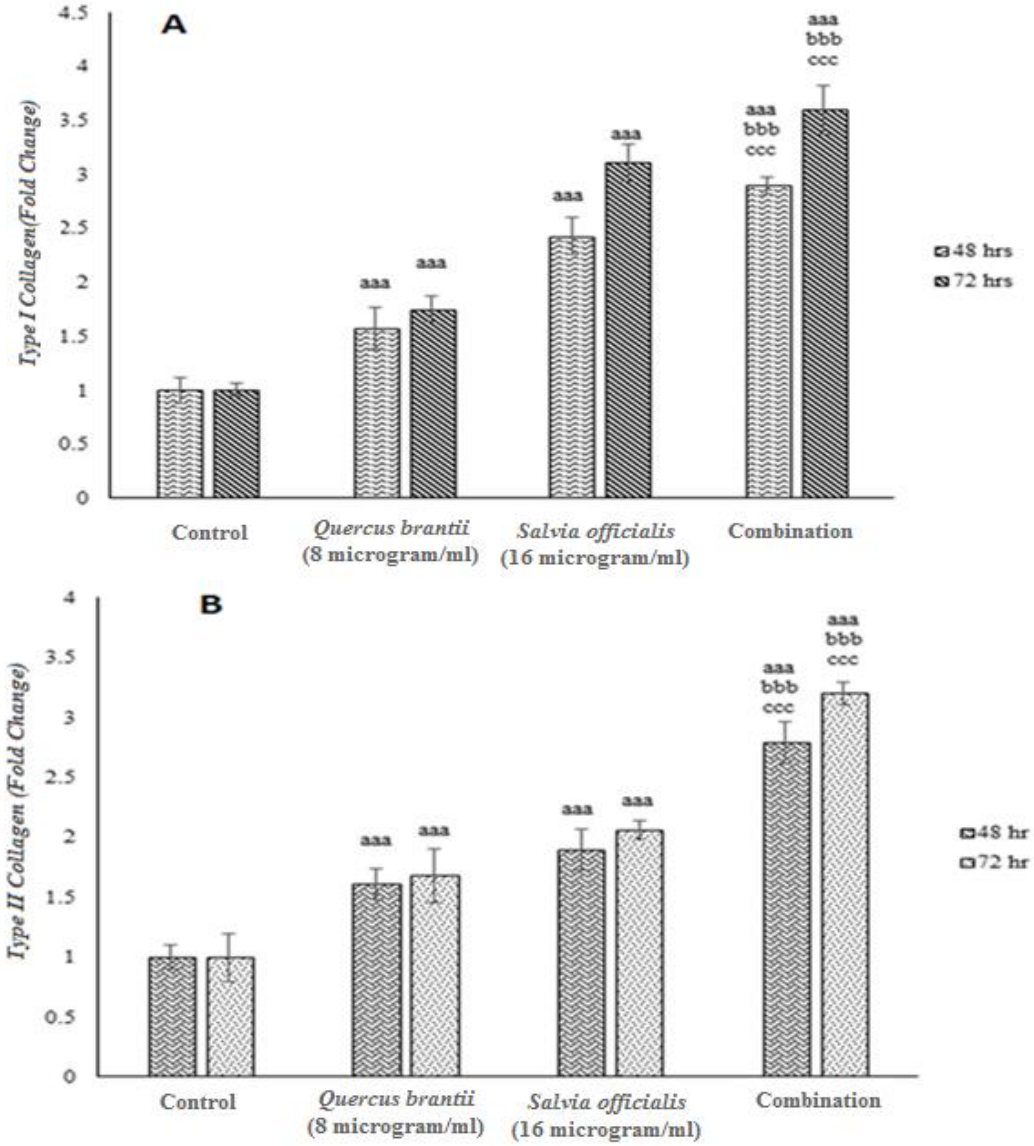


Figure 4. The effect of 8 and 16 µg/ml of *Quercus brantii* extract and *Salvia officinalis* extract, respectively, and the combination of two extracts on *collagen I* (A) and *collagen III* (B) expression levels in fibroblast cells after 48 and 72 hr of treatment. Control cells received the same volume of culture medium without extract. ^{a} indicates $p \leq 0.05$, ^{aa} $p \leq 0.01$ and, ^{aaa} $p \leq 0.001$ compared to the control group, ^{bbb} indicates $p \leq 0.001$ compared to *Quercus brantii* extract group, and ^{ccc} indicates $p \leq 0.001$ compared to *Salvia officinalis* extract group. All results are expressed as mean and SE obtained from three independent replicates for each treatment.

Discussion

In this study, the effect of the hydroalcoholic extracts of *Q. brantii* fruit and *S. officinalis*, alone and in combination, on the growth and migration potential of human fibroblast cells.

At first, to measure the effect of the extracts on the proliferation and survival of fibroblast cells, the MTT test was performed. The results showed that after 48 and 72 hr of treatment, *Q. brantii* fruit extract at a concentration of 8 µml caused a significant increase in cell proliferation compared to the control group ($p \leq 0.05$). Also, this extract had a toxic effect at concentrations of 128 and 256 µg/ml and significantly reduced cell survival compared to the control group ($p \leq 0.05$). In another study (10), the effect of hydroalcoholic extract of *Q. brantii* fruits on the survival of fibroblast cells (as a non-cancerous cell) and melanoma cells was measured. They used a constant concentration of 0.5 mg/ml and treatment periods of 1, 3, 6, 12, and 24 hr and showed that this extract had no significant effect on cell survival ($p \geq 0.05$). It is not possible to compare the results of this study with our study due to the difference in the concentrations used.

Also, our study showed that after 48 and 72 hr of *S. officinalis* extract treatment, the concentration of 16 µg/ml caused a significant increase in cell proliferation compared to the control group ($p \leq 0.05$). Also, this extract had no toxicity in the concentrations used compared to the control group. Valiyari et al. ~~Zare Shahneh et al~~. in 2013 investigated the inhibitory and cytotoxic activities of *S. officinalis* extract on human lymphoma and leukemia cells. In this study, the concentrations of 800, 600, 500, 400, 300, 200, 100, and 50 µg/ml and the treatment period of 12, 24, and 48 hr were used. Other studies ~~results~~ showed that hydroalcoholic extract at 50 to 800 µg/ml concentration had significant dose-dependent inhibitory effects on the proliferation of cancer cells. However, the extract did not cause any significant suppression in the proliferation of normal HUVEC cells. These results are in agreement with the results of our study that the extract did not show any significant inhibitory effect on the proliferation of non-cancerous fibroblast cells (11).

For combination treatment at 8 µg/ml concentration of *Q. brantii* fruits and 16 µg/ml of *S. officinalis* extracts were used for 48 and 72 hr, which had a stimulating effect on the proliferation of fibroblast cells. Our study indicated for the first time that the treatment with the combination of the two extracts significantly increased the proliferation of fibroblast cells more than each one alone ($p \leq 0.05$).

In the next step, the effect of the extracts alone and in combination on the migration of fibroblast cells was measured. The results showed that after 48 hr, the closure of the scratch was 16.01%, 18.76%, 25.15%, and 50.94% in the control group, *Q. brantii* fruits extract group, *S. officinalis* extract group, and combination of the two extracts group. After 72 hr, the closure of the scratch was 17.55%, 22.83%, 28.37%, and 68.94% in the control group, *Q. brantii* fruits extract group, *S. officinalis* extract group and the combination group. Keshavarz et al. (12) investigated the effect of *S. officinalis* extract on the migration of endothelial cells by scratch test. Their treatment period was 48 hr and the concentrations used were 100 and 200 µg/ml. Their results showed inhibition of migration with *S. officinalis* extract in a dose-dependent manner. These results showed that this extract inhibits HUVEC migration to fill the wound. The results of this study are in contrast with the present study, the reasons for which can be pointed to the difference in the type of cells and the concentrations. In our study, a concentration of 16 µg/ml was used. In our study, the effect of inducing cell migration by *Q. brantii* fruit extract and its combination with *S. officinalis* extract was reported for the first time.

The results of the ELISA test showed that *Q. brantii* fruits and *S. officinalis*

extracts after 48 and 72 hr caused a significant decrease in the concentration of TNF-α compared to the control group ($p \leq 0.05$) and the combination treatment had a greater reducing effect than each extract alone. After 48 and 72 hr, *Q. brantii* fruits and *S. officinalis* extracts alone did not have a significant effect on IL-6 level compared to the control group ($p \geq 0.05$), but the combination of the extracts significantly decreased IL-6 levels compared to the control group ($p \leq 0.05$). A study (13) measured the antioxidant and anti-inflammatory effects of *Q. brantii* fruit extract on ulcerative colitis in rats. Their results showed that treating rats with this extract decreased TNF-α and IL-6. Our study also showed a decrease in TNF-α and IL-6. The results of this study are in agreement with the present study. Kubatka et al. (14) investigated the oncostatic effects of *S. officinalis* extract in rodents and laboratory models of breast cancer. The results of this *in vivo* study showed that the serum levels of TGF-β (Transforming growth factor- beta) decreased significantly in cancer mice treated with the extract. The decrease in IL-6 and TNF-α level in the treated group was not significant compared to the control group. The results of this study are in contrast with the present study, the reasons for which can be pointed to the difference between the two studies and the investigated samples. In 2020, Farahpour et al. (8) investigated the effect of rapid wound healing with topical administration of *S. officinalis* essential oil on a wound model infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. They reported the increased VEGF mRNA levels following topical application of *S. officinalis* essential oil compared to the control group. The results of this study about VEGF are in contrast with the present study, the reasons for which can be pointed to the difference between the two studies and the investigated samples. Also, they reported that the expression levels of IL-6, IL-1β, and TNF-α were decreased in animals treated with essential oil on days 3, 7, and 14. These results confirm the results of our study.

The inflammatory phase is a critical component of the healing process, as it regulates the production of various cytokines and growth factors. Furthermore, the activation of the host immune system plays a vital role in combating infections; however, excessive inflammation can lead to tissue damage or multiple organ failure. During this phase, immune cells secrete pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in response to potential infections. Notably, a decrease in TNF-α level is associated with a reduction in other inflammatory cytokines, including IL-1, IL-6, IL-12, and IL-17. A similar relationship has been observed among TNF-α, IL-6, and IL-1β. Additionally, certain medicinal plants have been documented to enhance re-epithelialization in skin incision models by inhibiting the expression of TNF-α. (8).

Karimzadeh et al. (15) demonstrated that the topical application of *S. officinalis* extract enhances the distribution of fibroblasts. There exists an interrelationship between Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor-2 (FGF-2), wherein FGF-2 promotes angiogenesis, while the resultant VEGF plays a crucial role in sustaining the healing process (16). In our study, the effect of the combination of *Q. brantii* fruits and *S. officinalis* extracts on inflammatory factors and VEGF was reported for the first time.

The results of the real-time PCR test showed that *Q. brantii* fruit extract and *S. officinalis* extract after 48 and 72 hr caused a significant increase in collagen I and III expression compared to the control cells ($p \leq 0.05$) and combination treatment had a greater effect than each treatment alone.

Ebrahimi et al. (2012) conducted a study to evaluate the antibacterial and wound healing properties of methanolic extracts derived from the fruits of *Q. brantii* at three different concentrations (25, 50, and 75 mg/ml) in a rat model. The findings indicated that all tested concentrations

effectively inhibited the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*; however, the extracts at concentrations of 50 and 75 mg/ml demonstrated a statistically significant antibacterial effect. Furthermore, wounds treated with the extract exhibited enhanced epithelialization and wound contraction when compared to control wounds. The authors concluded that the fruits of *Q. brantii* contain bioactive compounds that possess both antibacterial and wound-healing properties (17). Khouzami et al. (2009) conducted a study to examine the gastroprotective properties of *Quercus infectoria* skin extract over a duration of two days. Their findings indicated a protective efficacy ranging from 56% to 67% against gastric lesions induced by ethanol (18). Khennouf et al. (2010) conducted an investigation into the anti-lipoperoxidation properties of various phenolic acids, flavonoids, and tannins extracted from *Quercus* species. The findings indicate that these purified compounds possess gastrointestinal protective effects, functioning as inhibitors of the lipid peroxidation process (19). These compounds demonstrated anti-lipoperoxidative activity, which is thought to contribute to the protective effects on digestion attributed to *Quercus* tannins and phenolic compounds. Furthermore, it has been documented that polyphenols, including tannic acid, quercetin, and ellagic acid found in the bark of *Q. infectoria*, possess the ability to inhibit the proton pump located in parietal cells, thereby leading to a reduction in gastric acid secretion (19). Khennouf et al. (2003) conducted a study in which they administered tannins extracted from the leaves of *Quercus suber* and *Quercus coccifera* to rats via oral ingestion. Their findings indicated that these tannins were effective in preventing gastric lesions induced by ethanol (20).

The hydroalcoholic extracts of *Q. brantii* fruits and *S. officinalis* alone can accelerate the wound healing process after 48 and 72 hr by increasing the proliferation of fibroblast cells, reducing inflammation, and increasing the expression of *collagen I* and *III*. Using the combination of both extracts, the observed effects were much higher than individual treatment. Taken together, the combination of these two extracts is suggested as a helpful strategy in wound healing.

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Conflicts of interest

There is no financial support and conflict of interest in this study.

Funding

This research received comprehensive support from the Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Iran.

Ethical Considerations The study has been fully endorsed and authorized by the scientific committee of Shahid Chamran University of Ahvaz.

Code of Ethics

The study has been fully endorsed and authorized by the scientific committee of Shahid Chamran University of Ahvaz.

Authors' Contributions

Vahid Hasanvand conducted the research, performed the investigation, carried out the formal analysis, curated the data, wrote the original draft, and prepared the visualizations. D Only the combination of *Q. brantii* and *S. officinalis* extracts at 8 and 16 µg/ml significantly increased cell proliferation.r. Mahnaz Kesmati supervised the project, provided resources, validated the findings, reviewed and edited the manuscript, and administered the project. Dr. Hossein Zhaleh and Dr. Elham Hoveizi supervised the work, contributed to

validation, and reviewed and edited the manuscript. All authors contributed to the conceptualization and design of the study.

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