



Wound Healing Activity of Extracts and Formulations of *Aloe vera*, Henna, *Adiantum capillus-veneris*, and Myrrh on Mouse Dermal Fibroblast Cells

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ABSTRACT

Background: Among the most important factors in wound healing pathways are transforming growth factor beta1 and vascular endothelial growth factor. Fibroblasts are the main cell in all phases wound closure. In this study, the extracts of plant materials such as *Adiantum capillus-veneris*, *Commiphora molmol*, *Aloe vera*, and henna and one mixture of them were used to treatment of normal mouse skin fibroblasts.

Methods: Cytotoxic effects of each extract and their mixture were assessed on mouse skin fibroblasts cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. We performed migration assays to assess migration properties of mouse skin fibroblasts cells in response to the extracts. Changes in the gene expression of the *Tgfb1* and *Vegf-A* genes were monitored by real-time polymerase chain reaction.

Results: *A. capillus-veneris*, *C. molmol* and henna extract improved the expression of *Tgfb1* gene. All used extracts upregulated the expression of *Vegf-A* gene and promoted the migration of mouse fibroblast cells *in vitro*.


Conclusions: The present study demonstrated that the mentioned herbal extracts might be effective in wound healing, through the improvement in the migration of fibroblast cells and regulating the gene expression of *Tgfb1* and *Vegf-A* genes in fibroblast cells treated with extracts.

Keywords: Fibroblast, herbal, transforming growth factor-β1, vascular endothelial growth factor, wound healing

INTRODUCTION

Wound healing process can be divided into four overlapping phases: Homeostasis, inflammation, proliferation, and

remodeling.^[1] The renovation of tissue veracity is due to cell-cell and cell-matrix interactions.^[2] These interactions are controlled by multiple cytokines and growth factors, as well as transforming growth factor-beta 1 (TGF-β1), and vascular endothelial growth factor (VEGF). Several cells such as platelets, macrophages, and T cells produce TGF-β1, which is an effective incentive of fibroblasts.^[3,4]

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TGF- β 1 recruits neutrophils and fibroblasts to the site of damage at the inflammatory phase of wound healing.^[5] TGF- β 1 suppresses severe inflammation and may therefore endorse the switch to a reparative phase. The effect of cytokine is almost never limited to one single phase of the healing progression. Fibroblasts are the main cell in all phases particularly, the proliferative phase of wound closure. TGF- β 1 also contributes to the migration, growth, diversity, and motivation of fibroblasts.^[6] TGF- β 1 stimulates fibroblasts, which differentiates into myofibroblasts. Fibroblasts collaborate with myofibroblasts to produce extracellular matrix (ECM), collagen^[7] and matrix proteins, such as fibronectin.^[8] TGF- β 1 is accompanied by VEGF and basic fibroblast growth factor which motivate angiogenesis.^[9]

VEGF-A (also known as VEGF) is created by several cells as well as endothelial cells, fibroblasts, smooth muscle cells, platelets, neutrophils, and macrophages.^[10]

Previous studies have recommended that VEGF plays an important role in angiogenesis, epithelization and collagen deposition during wound closure.^[2,11] In the remodeling step, fibroblasts deposit collagen and other ECM proteins modify the immature collagen matrix into mature scar tissue. VEGF can stimulate skin fibroblasts and promote scar tissue formation by means of various mechanisms.^[12,13]

Therapeutic plants have been utilized in different populations as remedial for injuries; hence, the advantage of it is their slight toxicity and availability.^[14] Several studies reported that herbal extracts can be utilized in the management of wound healing.^[15]

Aloe vera (Liliaceae) is a therapeutic herb that acts as a cathartic in food to remedy burns and wounds, and also contains antifungal, antimicrobial, antidiabetic, and hypoglycemic properties.^[16,17] *Commiphora molmol* (myrrh) is a plant that produces resin and contains antibacterial, antifungal, and antidiabetic properties.^[18] It has been utilized to tend wounds and also for intestinal disorders, diarrhea, coughs, inflammation, and chest ailments.^[19,20]

Adiantum capillus-vernus has a long history of medicinal use. It has anti-inflammatory, anti-diabetic, anti-infective, antimicrobial, and antioxidant properties.^[21] *A. capillus veneris* has significant angiogenic properties and improves wound healing *in vitro*.^[22] These properties indicate that local administration of *A. capillus-veneris* can decrease and heal wounds. Henna (*Lawsonia inermis*) is a well-known medicinal plant widely utilized to treat headaches, boils, diseases of the spleen, and skin disease.^[23] Experimental and clinical studies have reported that henna is an antibacterial and antifungal that supports wound healing.^[24]

Since the cells of mouse fibroblast cell line always have been to use it as model eukaryotic cells are similar

to human fibroblasts. In this study, cell lines C147 purchased from cell bank of Iran Pasteur Institute and the necessary tests were performed on it.

The aims of this study were to further explore the fibroblast proliferation and migration properties of these plant extracts and their mixture, to assess their wound healing activity by means of normal mouse skin fibroblasts.

METHODS

Collection of plant materials

Fresh leaves of *A. vera* were collected and identified from the botanic garden of Ahvaz Jondishapour University of Medical Sciences and the Department of Horticulture of the Faculty of Agriculture. A voucher specimen (No. 93) was deposited at the herbarium in the Faculty of Pharmacy. Shoots of *A. capillus-veneris* (Adiantaceae) were collected from Lorestan Province in Iran (no. 1661). Fresh henna leaves were collected from Kerman city in Iran (KF 1408). The oleo gum resin of *C. molmol* was obtained from Saudi Arabia. The origin of plant materials were systemically identified and approved at the herbarium of Shahid Chamran University of Ahvaz, Iran. After the collection of plants, fresh leaves of henna and *A. vera*, and the shoots of *A. capillus-veneris* were washed twist and dried at 60°C in an oven. The dried leaves and resin of the myrrh were then grinded in a blender into a fine powder.

Preparation of plant extracts

A total of 30g of powdered *A. vera* was macerated with ethanol at room temperature for 72 h, filtered through Whatman No. 1 paper filter, and then separated part was evaporated at 65°C in rotary until complete dryness.

Fifty grams of the powdered leaves of henna was soaked in 500 mL of 70% ethanol, macerated for 24 h and filtered (Whatman No. 185); the filtrate was then evaporated at 65°C in rotary evaporator until complete dryness.

Fifty grams of the powdered shoots of *A. capillus-veneris* was soaked in 300 mL of methanol at room temperature for 72 h and then filtered through with Whatman No. 1 filter paper. The filtrates were collected in separate flasks and were evaporated at 65°C in rotary evaporator until complete dryness.

Fifty grams of the dried powder of *C. molmol* oleo-gum-resin was soaked in 200 mL of methanol with continuous shaking for 24h at 40°C. The crude extracts were filtered by means of Whatman No. 1 filter paper. The filtrates were collected in separate flasks and were evaporated at 65°C in rotary evaporator until complete dryness. Dried extracts were powdered and kept at 4°C.

Cell culture

The normal mouse skin fibroblast line (c147) employed in this investigation were obtained from a National Cell

Bank of Iran, Pasteur Institute of Iran, Tehran, Iran and were cultured according to the source's guidelines. Fibroblast cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Biosera, France) + fetal bovine serum (FBS) 10% (Gibco, USA) medium, 100 U/mL penicillin and 100 µg/mL streptomycin (Bio-Idea, Iran). Cells were kept under standard culture conditions at 37°C and 5% CO₂. All cells were used between passages 5 and 6. Trypsin 0.025%-ethylenediaminetetraacetic acid 0.02% (Sigma-Aldrich, USA) in phosphate-buffered saline was used to separate fibroblast cells from the flasks.

Fibroblast 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays

The fibroblast cell viabilities and the cytotoxic effects of the each extract were scanned via the reduction of yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan by viable cells, in part by the action of dehydrogenase enzymes. Subsequently, intracellular formazan can be solubilized and distinguished by spectrophotometric means.^[25,26]

Six passages of fibroblast cells were trypsinized, suspended in RPMI + FBS 10%, and centrifuged. The supernatant was discarded and fibroblast cells were seeded (5×10^3 cells/well) in a 96 well plate, RPMI + FBS 10% (200 µL) was added and incubated in 5% CO₂ and 37°C for 24 h. All herbal extracts were liquefied in RPMI medium following filtration through a 0.2 µm filter to avoid cell contamination. About 50 µL of each extract and mixture of them in different concentrations were added to form a final concentration of 5, 50, 250, 500, 1000, 1500, and 2000 µg/mL.

Following the 24, 48 and 72 h incubation, the medium was replaced with 50 µL MTT (5 mg/mL) and incubated at 37°C for 4 h. The MTT was then discarded, and the formazan crystals were dissolved in 100 µL dimethyl sulfoxide (Bio-Idea, Iran). The optical density (OD) of cells was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad, USA).^[27]

This procedure was repeated in triplicate and to calculate the toxicity effect of each extract on fibroblast cell survival; the following formula was used:

In this formula, OD experimental and OD control represents the absorbance of treated cells and nontreated cells (control).

Cytotoxicity of extract % = [(OD control group – OD experimental group)/(OD control group)] × 100.

Cell migration assay

The *in vitro* scratch assay was utilized to detect the influence of each mentioned plant extracts and their mixture on the migration of mouse skin fibroblast cells. Fibroblasts were seeded at high density on a 24-well plate in RPMI 1640 medium containing 100 U/mL penicillin and 100 µg/mL streptomycin and 10% FBS. After 24 h, fibroblasts were attached and spread to form a confluent monolayer. Cell monolayer was scraped with a tip. Parted cells were removed and the attached cells were incubated with 500 µL of RPMI medium containing 5% FBS, 50 µg/mL of *A. vera*, *A. capillus-veneris* and *C. molmol* extract and 5 µg/mL of henna and 20 µg/mL of mixture of all above extracts was added and incubated at 37°C, 5% CO₂ and 90% humidity. Wound closure was examined by the quantity of transferred fibroblasts from the edge of the nick in extract treated wells in comparison to the control wells for 24, 48, and 72 h in four separate fields.^[28]

Expression analysis

Total RNA of fibroblast cells was isolated using TriReagent (Invitrogen). First strand complementary DNA (cDNA) was prepared by reverse transcription using PrimeScript™ RT Reagent kit (Takara, Japan) according to manufacturer instructions. The obtained cDNA was then used for real-time polymerase chain reaction (PCR) using master SYBR Green I (Takara Bio, Japan) on ABI 7900HT. Real-time PCR was executed at 95°C for 10 s, 62°C for 15 s, and 72°C for 8 s using the primers for the normalizing *Gapdh* gene against the *Tgfb1* and *Vegf-A* target genes. Primers were designed by Gen Script according to the cDNA sequences of mouse *Tgfb1*, *Vegf-A* and *Gapdh* in Gene Bank as shown in Table 1. Real-time PCR was performed in triplicate for every cDNA. Expression in fibroblast cells was treated with each extract and the mixtures at 24, 48, and 72 h after treatment were compared with the control (nontreated cells) after normalization with *Gapdh*.

We used relative gene expression, to identifying the increase or decrease of a transcript of target gene in treated sample versus control sample via normalizing with a housekeeping gene. To determine the difference of the gene expression between groups, the data were analyzed using the Relative Expression Software Tool (REST; version 2009). REST calculates the relative expression of

Table 1: Sequence of designed primers for each gene is shown as forward and reverse

| Gene | GenBank accession number | Forward primer (5'-3') | Reverse primer (5'-3') |
|---------------|--------------------------|------------------------|------------------------|
| GAPDH | NM_001289726.1 | ATGACTCTACCCACGGCAAG | CTGGAAGATGGTGTGGGTT |
| <i>Vegf-A</i> | NM_001287058.1 | GTCCTCACTTGGATCCCGACA | CCTGGCAGGCAAACAGACTTC |
| <i>Tgfb1</i> | NM_011577.1 | CTGAACCAAGGAGACGGAAT | GGTTCATGTCATGGATGGTG |

The primers used here for real time polymerase chain reaction were designed by www.GeneScript.com according their accession number

group means for target genes *Tgfβ1* and *Vegf-A* versus the normalizing *Gapdh* gene.

Statistical analysis

Statistical analysis was performed with SPSS (version 18) software. All data were presented as mean ± standard deviation. Kolmogorov–Smirnov test was utilized to examine parametric features of all statistics. One-way analysis of variance followed by Dunnett’s *post hoc* comparison was used for multiple between-group comparisons in MTT analysis. Student’s *t*-test was used to examine the difference in migration assay. To determine the difference of gene expression, the data were analyzed utilizing the REST; version 2009. REST calculates the relative expression of target genes *Tgfβ1* and *Vegf-A* versus the normalizing *Gapdh* gene. For all statistical tests, the level of statistical significance was set at $P < 0.05$. REST software performed the standard method called ΔCt analysis using the Ct values for each gene. The difference between the two ΔCt values $\Delta\Delta\text{Ct}$, represents the corrected shift of the target gene in treated sample versus control samples. This is a standard and published method and allowed us to determine orders of magnitude change. In the diagram, the relative changes in the expression of each gene expressed under the influence of herbal extracts were shown.

RESULTS

Fibroblasts 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The results of this study demonstrated significant ($P < 0.05$) difference in the toxicity of ethanolic extract of *A. vera* between doses 1000, 1500 and 2000 $\mu\text{g/mL}$ in comparison to the control (nontreated) cells, moreover, there was no significant ($P > 0.05$) difference at doses of 5, 50, 250 and 500 $\mu\text{g/mL}$, in comparison to the control cells at 24, 48, and 72 h after treatment [Figures 1-3 and Table 2].

Table 2: Cytotoxicity of ethanolic extract *Aloe vera*, after 24, 48 and 72 h treatment on mouse fibroblast cell line

| Toxicity of ethanolic extract of <i>Aloe vera</i> in comparison to control group (dose 0) | | | |
|---|-------|-------|-------|
| Dose ($\mu\text{g/mL}$) | P | | |
| | 24 h | 48 h | 72 h |
| 2000 | 0.000 | 0.000 | 0.000 |
| 1500 | 0.000 | 0.000 | 0.000 |
| 1000 | 0.026 | 0.000 | 0.000 |
| 500 | 0.900 | 0.142 | 0.999 |
| 250 | 0.943 | 1.000 | 0.000 |
| 50 | 0.728 | 1.000 | 0.000 |
| 5 | 1.000 | 0.813 | 0.000 |

$P < 0.05$ considered as a significant

The data showed significant ($P < 0.05$) difference in the toxicity of methanolic extract of *A. capillus-veneris* between doses 1000, 1500, and 2000 $\mu\text{g/mL}$ in comparison to the control cells, and there was no significant ($P > 0.05$) difference at doses of 5, 50, 250, and 500 $\mu\text{g/mL}$, in comparison to the cells group at 24, 48, and 72 h after treatment [Figures 1-3 and Table 3].

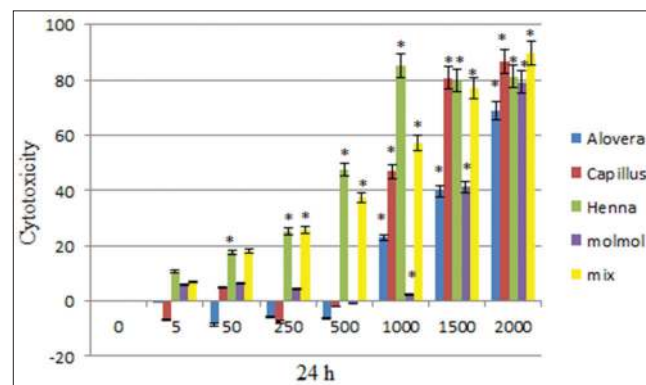


Figure 1: Cytotoxicity of *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of all extracts after 24 h treatment on mouse fibroblast cell line

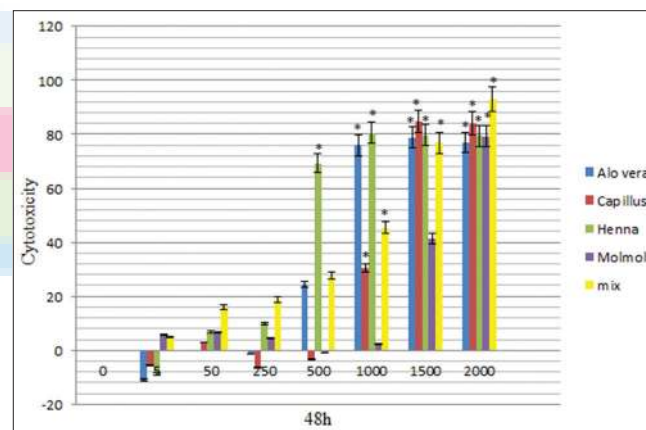


Figure 2: Cytotoxicity of *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of all extracts after 48 h treatment on mouse fibroblast cell line

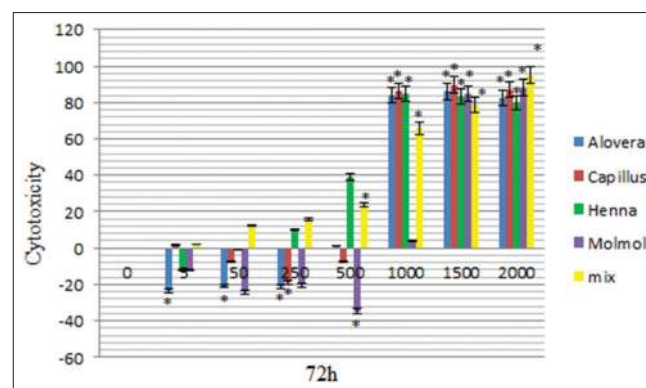


Figure 3: Cytotoxicity of *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of all extracts after 72 h treatment on mouse fibroblast cell line

There was a significant difference in the toxicity of methanolic extract of *C. molmol* between doses 1500 and 2000 µg/mL in comparison to the control cells, whereas there was no significant ($P > 0.05$) difference at doses at 5, 50, 250, 500, and 1000 µg/mL in comparison to the control cells at 24, 48, and 72 h after treatment [Figures 1-3 and Table 4].

There was a significant difference ($P < 0.05$) in the toxicity of ethanolic extract of henna between doses of 50, 250, 500, 1000, 1500, and 2000 µg/mL in comparison to control cells, but there was no significant ($P > 0.05$) difference at doses 5 µg/mL in comparison to the control cells at 24 h after treatment. There was a significant difference ($P < 0.05$) in the toxicity of ethanolic extract of henna between doses of 500, 1000, 1500, and 2000 µg/mL in comparison to control cells, but there was no significant ($P > 0.05$) difference at doses 5, 50, and 250 µg/mL in comparison to the control cells at 48, 72 h after treatment [Figures 1-3 and Table 5].

There is a significant differences ($P < 0.05$) in the the toxicity of mixture of all extracts between doses of

50, 250, 500, 1000, 1500, and 2000 µg/mL in comparison to control cells, there are no significant ($P > 0.05$) difference at doses 5 and 50 to the control cells at 24, 48, 72 h after treatment [Figures 1-3 and Table 6].

The 50% inhibitory concentration values of each used extracts for 24, 48, and 72 h treatment were calculated using linear regression as shown in Table 7. According to the results of the MTT test, the concentration of 50 µg/mL for *A. vera*, *A. capillus-veneris* and *C. molmol* extracts and 5 µg/mL for henna and 20 µg/mL of a mixture of all extracts that had low effects on cytotoxicity and fibroblast cells were selected.

Gene expression analysis

The present investigation determined changes in the expression of *Tgfb1* and *Vegf-A* genes by real-time-PCR in fibroblast cells with each extract and their mixture at 24, 48, and 72 h after treatment. Before data analysis, melting curves were obtained for each gene. The curves confirmed the accuracy of the peak corresponding

Table 3: Cytotoxicity of methanolic extract *Adiantum capillus-veneris*, after 24, 48 and 72 h treatment on mouse fibroblast cell line

| Toxicity of methanolic extract of <i>Adiantum capillus-veneris</i> in comparison to control group (dose 0) | | | |
|--|-------|-------|-------|
| Dose (µg/mL) | P | | |
| | 24 h | 48 h | 72 h |
| 2000 | 0.000 | 0.000 | 0.000 |
| 1500 | 0.000 | 0.000 | 0.000 |
| 1000 | 0.000 | 0.000 | 0.000 |
| 500 | 0.999 | 0.853 | 0.054 |
| 250 | 0.718 | 0.303 | 0.000 |
| 50 | 0.930 | 0.911 | 0.060 |
| 5 | 0.781 | 0.426 | 0.983 |

P<0.05 considered as a significant

Table 4: Cytotoxicity of methanolic extract *Commiphora molmol*, after 24, 48 and 72 h treatment on mouse fibroblast cell line

| Toxicity of methanolic extract of <i>Commiphora molmol</i> in comparison to control group (dose 0) | | | |
|--|-------|-------|-------|
| Dose (µg/mL) | P | | |
| | 24 h | 48 h | 72 h |
| 2000 | 0.000 | 0.002 | 0.000 |
| 1500 | 0.000 | 0.158 | 0.000 |
| 1000 | 1.000 | 0.728 | 0.999 |
| 500 | 1.000 | 0.992 | 0.028 |
| 250 | 0.976 | 0.446 | 0.297 |
| 50 | 0.860 | 0.999 | 0.165 |
| 5 | 0.920 | 1.000 | 0.787 |

P<0.05 considered as a significant

Table 5: Cytotoxicity of ethanolic extract henna, after 24, 48 and 72 h treatment on mouse fibroblast cell line

| Toxicity of ethanolic extract of henna in comparison to control group (dose 0) | | | |
|--|-------|-------|-------|
| Dose (µg/mL) | P | | |
| | 24 h | 48 h | 72 h |
| 2000 | 0.000 | 0.000 | 0.002 |
| 1500 | 0.000 | 0.000 | 0.002 |
| 1000 | 0.000 | 0.000 | 0.001 |
| 500 | 0.000 | 0.000 | 0.196 |
| 250 | 0.000 | 0.157 | 0.989 |
| 50 | 0.009 | 0.489 | 1.000 |
| 5 | 0.170 | 0.255 | 0.976 |

P<0.05 considered as a significant

Table 6: Cytotoxicity of mixture of *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts after 24, 48 and 72 h treatment on mouse fibroblast cell line

| Toxicity of the mixture of <i>Adiantum capillus-veneris</i> , <i>Aloe vera</i> , henna and <i>Commiphora molmol</i> extracts in comparison to control group (dose 0) | | | |
|--|-------|-------|-------|
| Dose (µg/mL) | P | | |
| | 24 h | 48 h | 72 h |
| 2000 | 0.000 | 0.005 | 0.045 |
| 1500 | 0.000 | 0.031 | 0.010 |
| 1000 | 0.028 | 0.000 | 00.00 |
| 500 | 0.000 | 0.078 | 00.00 |
| 250 | 0.000 | 0.509 | 0.367 |
| 50 | 0.107 | 0.875 | 0.068 |
| 5 | 0.340 | 0.270 | 1.00 |

P<0.05 considered as a significant

to the gene of interest and strings of primer dimer. A standard curve was plotted to evaluate the efficiency of the reaction using different dilutions of cDNA before performing real-time PCR. The relative expression of *Tgfβ1* and *Vegf-A* gene in fibroblast cells treated with the mentioned extracts and their mixture in comparison to control cells (nontreated) at 24, 48, and 72 h after treatment are shown in Figures 4 and 5.

Fibroblast migration assay

At the start of *in vitro* scratch test, there was little or no cells inside the scratch region as shown in Figure 6. Migration of fibroblast cell was improved after 72 h of treatment with an ethanolic extract of *A. vera* when compared to control ($P = 0.000$) and mixture of all other extracts ($P = 0.001$) [Figure 7]. Migration of Fibroblast cell was significantly ($P = 0.009$) improved after 72 h of treatment with methanolic extract of *C. molmol* compared to control and at 48h ($P = 0.049$) and 72 h ($P = 0.010$) treatment with mixture of all other extracts. Migration of fibroblast cell was significantly ($P = 0.000$) improved after 24 h of treatment with ethanolic extract of henna compared to control and at 24 h ($P = 0.002$) and 48 h ($P = 0.014$) treatment with mixture of all other extracts [Figure 7]. This study showed that there was no significant ($P > 0.05$) difference in the migration of fibroblast cell treated with methanolic extract of *A. capillus-veneris* compared to control cells or cells treated with mixture.

DISCUSSION

In recent years, the investigation on herbal treatment has improved worldwide. Various herbal extracts have shown beneficial properties as indicated in some studies.^[21,25] We examined *A. vera*, henna, *A. capillus-veneris* and *C. molmol* extracts and their mixture for treatment of normal mouse skin fibroblast line. It was demonstrated that methanolic extract of *A. capillus-veneris* and *C. molmol* and ethanolic extract of henna significantly improved the expression of *Tgfβ1* and *Vegf-A* genes at 48 h after treatment of fibroblast cells. Migration and proliferation of fibroblasts are essential during wound closure.^[29,30]

Interestingly, previous studies showed that nonhealing wounds often display a loss of TGF-β1 signaling.^[31,32] In the present study, significant up-regulation of the expression of *Tgfβ1* occurred in treated fibroblast cells through methanolic extract of *A. capillus-veneris*, *C. molmol* and ethanolic extract of henna in comparison to control cells at 48 h posttreatment.

Coppé *et al.*^[11] reported that hypoxia is a characteristic of wound that increases VEGF expression in different cells such as fibroblasts, keratinocytes, myocytes, and endothelial cells. Brem *et al.*^[33] reported that *in vitro* administration of VEGF encourages keratinocytes and fibroblasts cells migration, and increases wound closure. Romana-Souza *et al.*^[34] reported that VEGF and TGF-β1 increased the proliferation of keratinocytes *in vitro*.

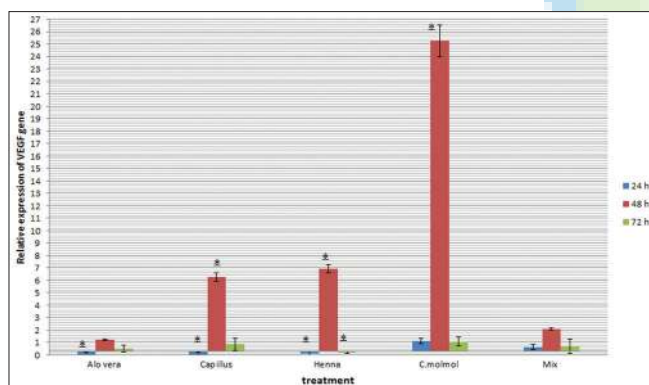


Figure 4: Changes in expression of *Vegf-A* gene in mouse fibroblast cells treated with *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of them after 24, 48 and 72 h (* $P < 0.05$ vs. control group)

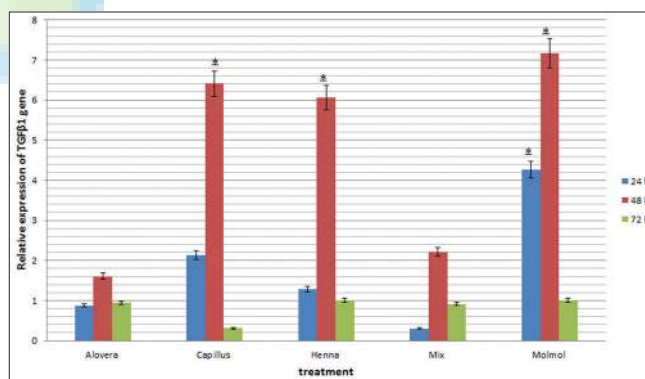


Figure 5: Changes in expression of *Tgfβ1* gene in mouse fibroblast cells treated with *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of them after 24, 48 and 72 h (* $P < 0.05$ vs. control group)

Table 7: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showing 50% inhibitory concentration of each extract at 24, 48 and 72 h after treatment

| Time (h) | Extract (μg/mL) | | | | |
|----------|------------------|-------|----------------------------------|--------------------------|-------------------------|
| | <i>Aloe vera</i> | Henna | <i>Adiantum capillus-veneris</i> | <i>Commiphora molmol</i> | Mixture of all extracts |
| 24 | 295.55 | 5.38 | 205.68 | 108.33 | 30.75 |
| 48 | 102.08 | 5.1 | 191.3 | 112.76 | 25.23 |
| 72 | 388.88 | 36 | 59.25 | 181.81 | 20.15 |

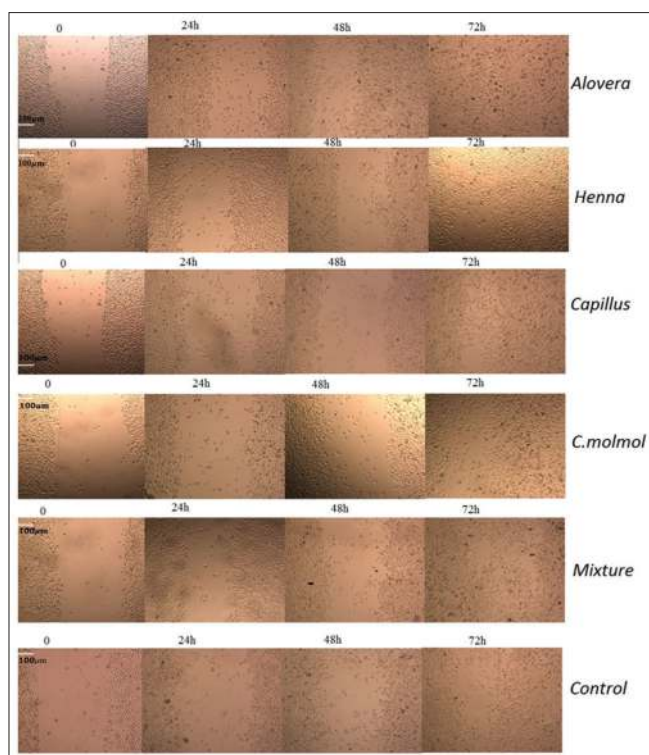


Figure 6: Migration of fibroblast cell at the start of *in vitro* scratch assay and after 24, 48 and 72 h of treatment with *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of them. Scale bar 100 μm

Cell migration is an extremely coordinated, multi-step course that organizes embryonic morphogenesis, tissue healing, and redevelopment.^[35,36] Fibroblasts migrate to the site of wound 48–72 h after injury. The migration of fibroblasts in the scratch area is likely to be a result of absolute cellular migration, proliferation, and cell death.^[28] We intended a wound healing assay to measure the influence of moistened extracts on fibroblast cells migration as one of the key steps in the healing process. Fibroblasts are very important during all stages of wound healing. Our study showed that fibroblast cells' migration was obviously increased when exposed to methanolic extract of *C. molmol* at 72 h compared to control cells and after 48 and 72 h compared to treated cells with mixture of herbal extracts. Previously, investigations have demonstrated that the properties of *C. molmol* can be attributed to terpenoids (exclusively furanoses quiterpenes), the active compounds existing in myrrh.^[20,37] Phenolic compounds, alkaloids and saponins have also been detected in extracts of *C. molmol*. Manjula *et al.* demonstrated that *C. molmol* resin has anti-inflammatory properties *in vitro* via inhibition of interferon- γ , interleukin-12 (IL-12), TNF- α , IL-1 β , and nitric oxide levels.^[38] Tipton *et al.*^[39] reported that myrrh oil have anti-inflammatory effects on human gingival fibroblasts and epithelial cells *in vitro*.

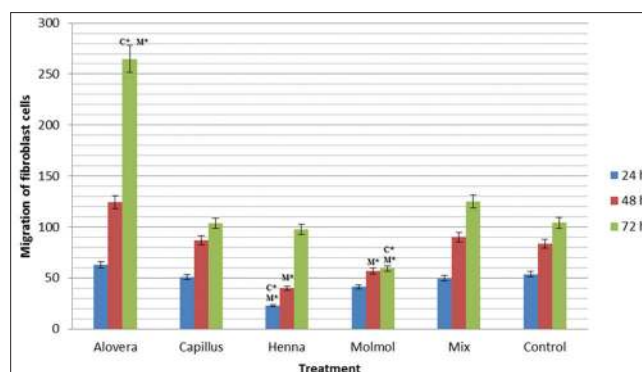


Figure 7: Migration of mouse fibroblast cells treated with *Aloe vera*, *Adiantum capillus-veneris*, henna, *Commiphora molmol* extracts and mixture of them after 24, 48 and 72 h

The migration assay significantly increased the migration of fibroblast cells after treatment with an ethanolic extract of henna at 24 h compared to control cells and at 24 and 48 h compared to treated cells with the mixture of herbal extracts. The leaves of the henna plant contain phytochemical ingredients such as tannin, gallic acid, glucose, mannitol, fat, resin, flavonoids, coumarins, and anthraquinones.^[40,41] Habbal *et al.* showed that henna leaf extracts are efficient in preventing infections by inhibiting the growth of microorganisms.^[42] It seems the strong cytotoxic properties of this extract could be attributed to its high antioxidant activities.

This study showed that there was no significant difference in the migration of fibroblast cell treated with methanolic extract of *A. capillus-veneris* compared to control cells or cells treated with the mixture of all other extracts. Nilforoushzhadeh *et al.* reported that *A. capillus-veneris* promoted angiogenic effects of endothelial cells and proliferation fibroblast cells *in vitro*.^[22] Antioxidant and anti-inflammatory activity of *A. capillus-veneris* could be attributed to polyphenolic and flavonoid activity.^[43-45]

Data showed that the migration assay obviously improved fibroblast cells' migration in exposure to ethanolic extract of *A. vera* at 72 h compared to control cells and herbal extract-treated cells. *A. vera* contains substantial amounts of phenol, saponin, and anthraquinones responsible for antibacterial, antiviral, and antifungal activity.^[46] Acemannan is the main carbohydrate element obtained from the *A. vera* leaf that has antiviral and anticancer effects and stimulates the immune system and macrophages.^[47] Jettanacheawchankit *et al.* investigated the influence of acemannan on the production of keratinocyte growth factor-1, VEGF, and Type I collagen production and reported that acemannan is important for oral wound healing.^[48]

Histological study revealed that *A. vera* enhances vascularity of the wound, which removes the dead tissue and increases the health of the wound. Collagen is the main extracellular protein in the homeostasis and

granulation tissue of a healing wound.^[49] The results obtained from this study revealed that ethanolic extract of *A. vera* had no significant effects on *Vegf-A* and *Tgfβ1* expression in fibroblast cells. *A. vera* leaves extract with high cytotoxicity effects could exhibit good *in vitro* antitumor activity. Kumar *et al.*^[50] showed that *A. vera* stimulated fibroblast proliferation and migration and that these properties of *A. vera* could help wound healing.

CONCLUSIONS

The results of this study showed that the mentioned herbal extract can be effective during wound healing. The aim of this study was not to isolate the composition of the extract and the role of each of them. Further studies for considering and confirming the *in vitro* properties of each of the mentioned extracts are necessary.

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Conflicts of interest

There are no conflicts of interest.

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