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 Tgfβ1 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 Tgfβ1 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 Tgfβ1 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]
TGF-β1 recruits neutrophils and fibroblasts to the site of damage at the inflammatory phase of wound healing.\textsuperscript{[5]} TGF-β1 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-β1 also contributes to the migration, growth, diversity, and motivation of fibroblasts.\textsuperscript{[6]} TGF-β1 stimulates fibroblasts, which differentiates into myofibroblasts. Fibroblasts collaborate with myofibroblasts to produce extracellular matrix (ECM), collagen\textsuperscript{[7]} and matrix proteins, such as fibronectin.\textsuperscript{[8]} TGF-β1 is accompanied by VEGF and basic fibroblast growth factor which motivate angiogenesis.\textsuperscript{[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.\textsuperscript{[10]}

Previous studies have recommended that VEGF plays an important role in angiogenesis, epithelization and collagen deposition during wound closure.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[14]} Several studies reported that herbal extracts can be utilized in the management of wound healing.\textsuperscript{[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, anti-diabetic, and hypoglycemic properties.\textsuperscript{[16,17]} Commiphora molmol (myrrh) is a plant that produces resin and contains antibacterial, antifungal, and anti-diabetic properties.\textsuperscript{[18]} It has been utilized to tend wounds and also for intestinal disorders, diarrhea, coughs, inflammation, and chest ailments.\textsuperscript{[19,20]}

Adiantum capillus-vernis has a long history of medicinal use. It has anti-inflammatory, anti-diabetic, anti-infective, antimicrobial, and antioxidant properties.\textsuperscript{[21]} A. capillus-vernis has significant angiogenic properties and improves wound healing \textit{in vitro}.\textsuperscript{[22]} These properties indicate that local administration of A. capillus-vernis 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.\textsuperscript{[23]} Experimental and clinical studies have reported that henna is an antibacterial and antifungal that supports wound healing.\textsuperscript{[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 \textit{A. vera} were collected and identified from the botanic garden of Ahvaz Jondishapur 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 \textit{A. capillus-vernis} (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 \textit{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 \textit{A. vera}, and the shoots of \textit{A. capillus-vernis} 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 \textit{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 \textit{A. capillus-vernis} 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 \textit{C. molmol} oleo-gum-resin was soaked in 200 mL of methanol with continuous shaking for 24 h 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 (Biocera, 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 × 10⁵ cells/well) in a 96 well plate, cells/well) in a 96 well plate, 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 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.[25]

**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 Tgfβ1 and Vegf-A target genes. Primers were designed by Gen Script according to the cDNA sequences of mouse Tgfβ1, 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

| Gene | GenBank accession number | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|------|--------------------------|------------------------|-----------------------|
| GAPDH | NM_001289726.1           | ATGACTCTACCAGCGAAG     | GTGAGAATGCATTTATGGTT  |
| Vegf-A | NM_0012897058.1         | GTCTCTACCTGTCCGACA     | CCTGGCAGCACAAGAAGCTTC |
| Tgfβ1 | NM_011577.1              | CTGAACCAAGGAGCGAAT     | GGTTCATGTCAAGATGGTG  |

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 ∆Ct analysis using the Ct values for each gene. The difference between the two ∆Ct values ∆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 µg/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 µg/mL, in comparison to the control cells at 24, 48, and 72 h after treatment [Figures 1-3 and Table 2].

| Toxicity of ethanolic extract of Aloe vera in comparison to control group (dose 0) | 24 h | 48 h | 72 h |
|---|---|---|---|
| Dose (µg/mL) | P | | |
| 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 µg/mL in comparison to the control cells, and there was no significant (P > 0.05) difference at doses of 5, 50, 250, and 500 µg/mL, in comparison to the cells group at 24, 48, and 72 h after treatment [Figures 1-3 and Table 3].
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 4].

There is a significant differences (*P < 0.05*) in the in 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 4].

Gene expression analysis

The present investigation determined changes in the expression of *Tgfβ1* 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 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) | Toxicity of ethanolic extract of henna in comparison to control group (dose 0) | P |
| 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 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 *Adiantum capillus-veneris*, Aloe vera, henna and *Commiphora molmol* extracts in comparison to control group (dose 0) |
| Dose (µg/mL) | Toxicity of the mixture of *Adiantum capillus-veneris*, Aloe vera, henna and *Commiphora molmol* extracts in comparison to control group (dose 0) | P |
| 2000 | 0.000 | 0.005 | 0.045 |
| 1500 | 0.000 | 0.031 | 0.010 |
| 1000 | 0.028 | 0.000 | 0.000 |
| 500 | 0.000 | 0.078 | 0.000 |
| 250 | 0.000 | 0.509 | 0.367 |
| 5 | 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.

**DISCUSSION**

In recent years, the investigation on herbal treatment has improved worldwide. Various herbal extracts have shown beneficial properties as indicated in some studies. 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.

Interestingly, previous studies showed that nonhealing wounds often display a loss of TGF-β1 signaling. In the present study, significant up-regulation of the expression of Tgfβ1occurred 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.
Cell migration is an extremely coordinated, multi-step course that organizes embryonic morphogenesis, tissue healing, and redevelopment.[13,36] 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 the 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-1β, 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.

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 cells treated with methanolic extract of A. capillus-veneris compared to control cells or cells treated with the mixture of all other extracts. Nilforoushzadeh 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. The results obtained from this study revealed that ethanolic extract of A. vera had no significant effects on 

**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.

**Acknowledgments**

The authors hereby express thanks to the Shahid Chamran University of Ahvaz for providing grant for this study. The authors would like to thank Narges Laboratory in Khuzestan, Iran, for technical assistance.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**Received:** 24 Sep 16  **Accepted:** 22 Jan 17  **Published:** 10 Mar 17

**REFERENCES**

1. Deonarine K, Panelli MC, Stashower ME, Jin P, Smith K, Slade HB, et al. Gene expression profiling of cutaneous wound healing. J Tra Nat Med 2007;5:11.
2. Olczyk P, Mercner L, Komosinska-vasseva K. The role of the extracellular matrix components in cutaneous wound healing. Biomed Res Int 2014;2014:747584.
3. Blaktyny R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 2006;23:594-608.
4. Darby IA, Bisucci T, Hewitson TD, MacLellan DG. Apoptosis is increased in a model of diabetes-impaired wound healing in genetically diabetic mice. Int J Biochem Cell Biol 1997;29:191-200.
5. Khali N, Greenberg AH. The role of TGF-beta in pulmonary fibrosis. Ciba Found Symp 1991;157:194-207.
6. Nirodi CS, Devalara J, Nannay MB, Arrindell S, Russell JS, Trupin J, et al. Chemokine and chemokine receptor expression in keloid and normal fibroblasts. Wound Repair Regen 2000;8:371-82.
7. Werner S, Krieg T, Smola H. Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol 2007;127:998-1008.
8. Maroni D, Davis JS. Transforming growth factor Beta 1 stimulates profibrotic activities of luteal fibroblasts in cows. Biol Reprod 2012;87:127.
9. Khahehi I, Keshavarz S, Imani Fooladi AA, Ebrahim M, Yazdani S, Panahi Y, et al. Loss of expression of TGF-βs and their receptors in chronic skin lesions induced by sulfur mustard as compared with chronic contact dermatitis patients. BMC Dermatol 2011:11:2.
10. Bao P, Kodra A, Tomic-Canic M, Galinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res 2009;153:347-58.
11. Coppé JP, Kauser K, Campisi J. Beauséjour CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem 2006;281:29568-74.
37. Dolara P, Corte B, Ghezziare C, Pugliese AM, Corbasi E, Menichetti S, et al. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med 2000;66:356-8.

38. Manjula N, Gayathri B, Vinaykumar KS, Shankernarayanan NP, Vishwakarma RA, Balakrishnan A. Inhibition of MAP kinases by crude extract and pure compound isolated from Commiphora mukul leads to down regulation of TNF-alpha, IL-1beta and IL-2. Int Immunopharmacol 2006;6:122-32.

39. Tipton DA, Lyle B, Babich H, Dabbous MKh. In vitro cytotoxic and anti-inflammatory effects of myrrh oil on human gingival fibroblasts and epithelial cells. Toxicol In Vitro 2003;17:301-10.

40. Kirkland D, Marzin D. An assessment of the genotoxicity of 2-hydroxy-1,4-naphthoquinone, the natural dye ingredient of Henna. Mutat Res 2003;537:183-99.

41. Jeyaseelan EC, Jenothiny S, Pathmanathan MK, Jeyadevan JP. Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of Lawsonia inermis L. from Jaffna. Asian Pac J Trop Biomed 2012;2:798-802.

42. Habbal O, Hasson SS, El-Hag AH, Al-Mahroooqi Z, Al-Hashmi N, Al-Bimani Z, et al. Antibacterial activity of Lawsonia inermis Linn (Henna) against Pseudomonas aeruginosa. Asian Pac J Trop Biomed 2011;1:173-6.

43. Askari G, Ghiasvand R, Feizi A, Ghanadian SM, Karimian J. The effect of quercetin supplementation on selected markers of inflammation and oxidative stress. J Res Med Sci 2012;17:637-41.

44. Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem 2011;11:298-344.

45. Ibraheim ZZ, Ahmed AS, Gouda YG. Phytochemical and biological studies of Adiantum copilus-veneris L. Saudi Pharm J 2011;19:65-74.

46. Choi SW, Son BW, Son YS, Park YI, Lee SK, Chung MH. The wound-healing effect of a glycoprotein fraction isolated from Aloe vera. Br J Dermatol 2001;145:535-45.

47. Tanaka M, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, et al. Identification of five phytosterols from Aloe vera gel as anti-diabetic compounds. Biol Pharm Bull 2006;29:1418-22.

48. Jettanachewchankanit S, Sasithanasate S, Sangvanich P, Banlunara W, Thunyakitsipal P. Acemannan stimulates gingival fibroblast proliferation; expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and type I collagen; and wound healing. J Pharmacol Sci 2009;109:525-31.

49. Morgan C, Nigam Y. Naturally derived factors and their role in the promotion of angiogenesis for the healing of chronic wounds. Angiogenesis 2013;16:493-502.

50. Kumar MS, Datta PK, Dutta Gupta S. In vitro evaluation of UV opacity potential of Aloe vera L. gel from different germplasms. J Nat Med 2009;63:195-9.