Journal of Research in Medical and Dental Science
2018, Volume 6, Issue 3, Page No: 230-236

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Evaluation of wound healing activities of pomegranate (Punica granatum - Lythraceae) peel and pulp

Morteza Sheikh Asadi[1], Sayid Mahdi Mirghazanfari[2]*, Masumeh Dadpay[3], Ehsan Nassireslami[4]

[1] Researcher at AJA University of Medical Sciences, Tehran, Iran.
[2] Department of Physiology and Iranian Medicine, School of Medicine, AJA University of Medical Sciences, Tehran, Iran
[3] Department of Pathology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran
[4] Department of Pharmacology & Toxicology, AJA University of Medical Sciences, Tehran, Iran

DOI: 10.24896/jrmds.20186336

ABSTRACT

Background: Wound healing is an active and complex process. Wounds cause serious problems in many cases, while there is no fast and reliable cure method. Numerous medicines, mostly as ointments, have been used to treat wounds. However, they can have some defects, limitations, and side effects. Therefore, exploration for new natural compounds for curing wounds is important. Traditional Iranian Medicine (TIM) is becoming popular, which can return patients to health with minimal side effects. Pomegranate is well reported for its medicinal properties. In TIM, Punica granatum fruits have been used to treat inflammatory disorders and wounds. Methods: In this study, 60 Wistar rats (male, 180±10 g) were used. The rats were randomly divided into five groups (each containing 12), including Eucerin as control; phenytoin as standard drug; the top layer of pomegranate peel (peel), pomegranate pulp (pulp), and peel + pulp as investigation groups. The back skin of deeply-unconscious animals was wounded (2 cm long). For 14 days from wound establishment, the wounds were treated topically with the medicines above. Wound length and improvement percentage were investigated by analyzing images of wounds. Three rats of each group were killed on days 3,7,10 and 14, and then the wounded skin were cut at a certain area for pathological studies. Epithelization; neovascularization; fibroblast, PMN and macrophage count were investigated. Results: All groups had significant (P<0.01) wound healing in comparison with the control group on all days, but phenytoin and pulp were the most effective. Phenytoin and peel significantly improved some histopathological parameters (P<0.05). Conclusion: Various parts of the pomegranate skin have different effects on wound healing. The extract of the top layer of the peel significantly improved the wound healing process, whereas that of the pulp showed no promising effects. Accordingly, the top layer of the peel is suggested for further studies on wound healing.

Key words: Traditional Iranian Medicine, Medicinal Plants, Punica Granatum, Wound Healing.

HOW TO CITE THIS ARTICLE: Morteza Sheikh Asadi, Sayid Mahdi Mirghazanfari, Masumeh Dadpay, Ehsan Nassireslami, Evaluation of wound healing activities of pomegranate (Punica granatum - Lythraceae) peel and pulp, J Res Med Dent Sci, 2018, 6 (3): 230-236, DOI: 10.24896/jrmds.20186336

***Corresponding author**: Sayid Mahdi Mirghazanfari
e-mail: smmirghazanfari@ajaums.ac.ir
Received: 15/01/2018
Accepted: 10/03/2018

INTRODUCTION

After cancer and cardiovascular diseases, wounds are the third costliest disorders [1]. In order for a wound to be healed, and to rebuild tissues damaged by physical, chemical, and pathogenic organisms such as bacteria and viruses, the process of wound healing is necessary [2]. Wound healing is a complex pathophysiological process, which includes multiple cellular and biochemical reactions including inflammation; neovascularization; and collagen deposition [3]. New therapeutic methods to achieve accelerated wound healing, prevention of wound infection, increased elasticity of the wound bed, and decreased volume of scar tissue surrounding the wound are always being studied [4]. Therefore, many scholars carry out investigations focused on

wound healing, results of which are the introduction of new herbal and chemical ointments, although none has been recommended as a truly effective medicine [5].

Chemical medicines used for wound healing have their own drawbacks. For example, betadine generally has positive effects, but there are also reports that it has delayed wound healing, due to the death of fibroblasts and keratinocytes as well as the suppression of lymphocytes [6]. Undesirable and unwanted effects of chemical drugs has led today's physicians to apply therapeutic methods using natural and biological materials. Accordingly, medicines and antibiotics that are plant-based are becoming more popular, so multiple centers have started working in this field [7].

More than 90 percent of people in developed countries believe medicinal plants are the reliable sources for their primary health care needs [8]. Pomegranate (P. granatum, Lythraceae) is an important medicinal plant, to which many properties have long been attributed. In Traditional Indian Medicine, people considered pomegranate to be a pharmacy on its own. They used the bark and the roots as anthelmintic agents. The fruit skin is a strong astringent, which was used to treat diarrhea, while the juice was utilized as an antifever and tonic agent [9]. In Traditional Greek Medicine, pomegranate flowers were used to treat diabetes [10]. Nowadays, numerous articles are being published on the medicinal properties of pomegranate including antioxidant, anticarcinogenic, and anti-inflammatory effects. The studies are mostly focused on prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage as well as infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity [11].

There is no comparative report on the medicinal effects of various parts of the pomegranate fruit. Considering the multiple medicinal properties of the fruit and the different medicinal components of the peel and pulp, the present study was, for the first time, carried out on the effects of the top layer of the peel (peel) and the pulp on wound healing in rats.

MATERIALS AND METHODS

Animals and keeping conditions

In this study, 60 male rats (180 ± 10 g) were used. The rats were kept at standard conditions: 23 ± 1 °C; 12/12 hours light/dark. During the keeping period, the food was served as special plates. There were no limitations regarding food or water [12].

Wounding and treatments

The experimental rats were anesthetized by intra-peritoneal injection of thiopentone (25 mg/kg, i.p.). Their backs were shaved and then cleaned with 70% ethanol. A full-thickness incision was made 2 cm apart from the dorsal region, on the right. After being recovered from anesthesia, the rats were housed in sterile cages, each in one. The rats were randomly separated into 5 groups (12 rats in each group): group 1 (Eucerin); group 2 (Phenytoin); group 3: hydroalcoholic extract of the top layer of pomegranate peel (peel); group 4: hydroalcoholic extract of pomegranate pulp (the membrane between the arils: Pulp); and group 5: hydroalcoholic extract of the top layer of pomegranate (peel + pulp). The treatments were applied topically twice a day. 3 rats from each group in days 3, 7, 10 and 14 (from wound establishment) were used for wound healing and histopathologic studies.

Preparation of hydroalcoholic extract

Fruit peel, pulp, and arils were carefully separated. Maceration was used for the preparation of the extract. 250 g shade-dried sample was powdered, to which 2.5 L ethanol: water (3:1) was added. The mixture was shaken at ambient temperature for 72 h. After that, the extract was filtered, concentrated as much as possible using a rotary device (at 60 RPM and 45 °C), and then dried at ambient temperature.

Wound Healing

Wound healing process was studied from just after the wounds were created until their complete healing. In order to measure wound healing percentage, wounds photos were taken. The distance of camera from skin was fix in all cases. Video Image Analyze software was then used to compute the lengths of the wounds, and wound healing percentage on different days was calculated according to the following equations:

$$\text{Wound size percentage} = \frac{\text{Wound area on day X}}{\text{Wound area on day 0}} \times 100$$

Wound healing percentage in day X = 100 -

Histological examinations

The wound (and the surrounding tissue) of each rat was excised on the experimental days and then fixed in 10% formalin. 5 µm-thick sections were stained according to hematoxylin-eosin (H&E) method [13], to create microscopic slides. The slides were then photographed using a modern microscope (Olympus-BX51BX51, America, Center Valley PA) at ×200. Scale of epithelialization; neovascularization and fibroblast, macrophage and PMN (polymorphonuclear cells) proliferation were scored as zero (-), minimal (+), slight (++), moderate (+++) and severe (++++), according to Süntar et al. [14].

Data analysis

Data were recorded and shown as mean ± SE and analyzed by one-way analysis of variance and Tuckey test. P-values lower than 0.05 were considered significant.

RESULTS

Wound healing percentage

The results for healing process are shown in Fig. 1. All interventional groups had a significant difference from eucerin's on every experimental day except pulp on day 3. In each day of the study, groups pulp and pulp+peel were significantly different from phenytoin's. The healing percentage data on every experimental day are shown in Table 1.

Histopathology

The histopathological results are presented in tables 1-4. On day 3, there was no significant difference between the groups (Table 2). On days 7, 10, and 14, there was significant difference in some histopathological parameters between some experimental groups as shown in Tables 3, 4, and 5.

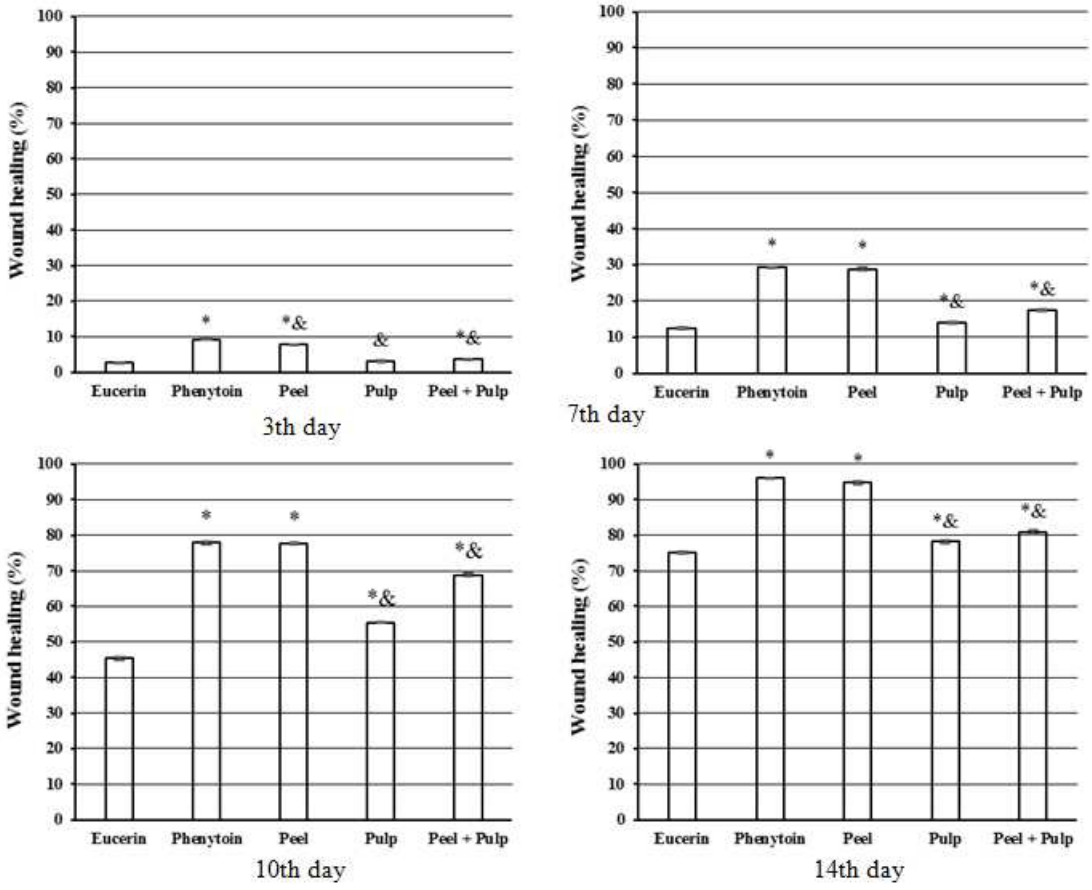

Fig 1. Comparison of wound healing percentages in different experimental groups on each experimental day. Data are shown as means ± SE. *: significant difference with eucerin (P< 0.01). &: significant difference with phenytoin (P< 0.01).

Table 1. Comparison of wound healing percentages in different experimental groups on each experimental day. Data are shown as means ± SE. *: significant difference with eucerin (P< 0.01). &: significant difference with phenytoin (P< 0.01).

	Post wounding days			
Experimental groups	**3th day**	**7th day**	**10th day**	**14 day**
Eucerin	2.67 ± 17	12.33 ± 0.33	45.33 ± 0.44	75.17 ± 0.33
Phenytoin	9.17 ± 17 *	29.33 ± 0.17 *	77.83 ± 0.60 *	96.00 ± 0.29 *
peel	7.83 ± 17 *&	28.83 ± 0.44 *	77.67 ± 0.17 *	94.83 ± 0.44 *
Pulp	3.00 ± 29 *	14.00 ±0.29 *&	55.50 ± 0.29 *&	78.17 ± 0.60 *&
Peel + Pulp	3.67 ± 17 *&	17.50 ± 0.29 *&	68.83 ± 0.44 *&	80.83 ± 0.73 *&

Table 2. Histopathological parameters on days 3. Scale of parameters were scored as zero (-), minimal (+), slight (++), moderate (+++) and severe (++++).

Experimental groups	**Epithelialization**	**Fibroblast**	**Neovascularization**	**Polymorphonuclear cells (PMN)**	Macrophage
Eucerin	**+**	**-**	**-**	**+++**	**+++**
Phenytoin	**++**	**+**	**+**	**+**	**++**
Peel	**++**	**+**	**+**	**+**	**++**
Pulp	**+**	**-**	**-**	**++**	**++**
Peel + Pulp	**++**	**+**	**+**	**++**	**++**

Table 3. Histopathological parameters on days 7. Scale of parameters were scored as zero (-), minimal (+), slight (++), moderate (+++) and severe (++++). *: significant difference with eucerin (P< 0.05). €: significant difference with eucerin (P< 0.01). &: significant difference with phenytoin (P< 0.05).

Experimental groups	**Epithelialization**	**Fibroblast**	**Neovascularization**	**Polymorphonuclear cells (PMN)**	Macrophage
Eucerin	**++**	**+**	**+**	**++**	**++++**
Phenytoin	**+++**	**++** *	**++**	**+++**	**+++**
Peel	**++++***	**++** *	**++**	**+++**	**+++**
Pulp	**++**	**+** &	**+**	**++**	**++** &
Peel + Pulp	**+++**	**+** &	**++**	**++**	**++** &

Table 4. Histopathological parameters on days 10. Scale of parameters were scored as zero (-), minimal (+), slight (++), moderate (+++) and severe (++++). €: significant difference with eucerin (P< 0.01). &: significant difference with phenytoin (P< 0.01).

Experimental groups	**Epithelialization**	**Fibroblast**	**Neovascularization**	**Polymorphonuclear cells (PMN)**	Macrophage
Eucerin	**++**	**++**	**++**	**+**	**++**
Phenytoin	**++++** €	**++++** €	**+++**	**++**	**+**
Peel	**+++** &€	**++++** €	**+++**	**++**	**+**
Pulp	**++** &	**++** &	**++**	**+**	**+**
Peel + Pulp	**++** &	**++** &	**++**	**+**	**++**

Table 5. Histopathological parameters on days 14. Scale of parameters were scored as zero (-), minimal (+), slight (++), moderate (+++) and severe (++++). €: significant difference with eucerin (P< 0.01). &: significant difference with phenytoin (P< 0.01).

Experimental groups	**Epithelialization**	**Fibroblast**	**Neovascularization**	**Polymorphonuclear cells (PMN)**	Macrophage
Eucerin	**++**	**+**	**+**	**+**	**+**
Phenytoin	**+++**	**+++** €	**++**	**-** €	**-** €
Peel	**+++**	**+++** €	**++**	**-** €	**-** €
Pulp	**++**	**+** &	**+**	**+** &	**+** &
Peel + Pulp	**++**	**++** €&	**++**	**-** €	**-** €

DISCUSSION

This study reveals that the herbal medicines used for wound healing can be helpful. However, the different plant parts have very different effects. For example, the extract of the top layer of peel was very effective in wound healing, while that of pulp was not that promising. The ointments made of the extract of peel or the extracts of both peel and pulp (to a lesser extent) were effective in improving the visual attributes of the wounds and general health of the animals as well as in histopathological parameters. Regarding wound healing, the best results were achieved in Phenytoin and Peel. Regarding pathological parameters, there were significant differences

among the experimental groups. Overall, the groups were significantly different respecting histopathological parameters. Phenytoin and Peel significantly differed from the other groups when wound condition was considered.

Wound contraction and connective tissue deposition reduce wound surface area. With their contractive properties, fibroblasts cells make epidermis layer shrink, thereby decreasing wound dimensions [15]. Of active factors in wound healing are antioxidants, which pomegranate is full of [16].

Reactive oxygen species (ROS) damage cells and tissues, hence bad for wound healing [17]. The ROS proxidize lipids, inactivate enzymes, and break DNA down. Studies have shown that vitamin C [18] and vitamin E [19] are effective antioxidants to remove free radicals. The antioxidant and anti-inflammatory properties of pomegranate fruits, which can accelerate wound healing process, can be attributed partly to these vitamins. Besides, tannins and polyphenols like ellagic acid, gallic acid, and coumaric acid are found in pomegranate fruits [20-22]. Punicalagin and Punicalin were also shown to have anti-inflammatory, hepatoprotective, and antigenotoxic properties [23].

We, for the first time, demonstrated that the extract of the top layer of the peel is much more effective than that of the pulp in wound healing. Studies have proven that pomegranate peel shows much more antioxidant properties that do the pulp, which is due to their different contents of phenolics, flavonoids, and proathocyanidins [24-26]. In a study by Li et al. [25], phenolics, flavonoids and proathocyanidins in the peel were observed to be 10.2, 3.4, and 2 times as those in the pulp, respectively.

The wound healing results of the present study are in agreement with those (Abu-Al-Basal [27] on Rosmarinus officinalis; Heersaiy et al. [28] on Borago Officinalis; Tümen et al. [29] on Pinus pinaster Ait; OZBİLGİN et al., [30] on Tanacetum) reported in literature. Many medicinal plants effective in wound healing have a similar property: a high content of phenolics. These phytochemicals show a wide range of reactions to oxygen-containing compounds and macromolecules, making free radicals nitrogenated [31]. Of the reasons for the wound healing properties of the pomegranate peel is the presence of phenolics. It is well documented that some phenolic compounds increase the expression of the gene responsible for the production of connective tissue, while they suppress the gene expression of the regulator of collagen type 1. These are followed by myofibroblast production and differentiation, growth of connective tissue, and regimentation of collagens [32].

The results of all of these studies indicate the positive effects of these plants in the reduction of inflammation and hence in the acceleration of wound healing process. Prudente et al. (2013) showed that herbal extracts accelerate the inflammation stage and migration of inflammatory cells [33].

Overall, it could be concluded that the extract of pomegranate peel accelerates wound healing by reducing the number of immune cells, accelerating the second stage of the healing, and accelerating the migration of fibroblast to the wounded tissue. Fibroblasts produce collagens, elastin, and proteoglycans. Collagens increase the elasticity of the wound, thereby making it contracted [34].

CONCLUSION

Various parts of the pomegranate skin have different effects on wound healing. The extract of the top layer of the peel significantly improved the wound healing process, whereas that of the pulp showed no promising effects. Accordingly, the top layer of the peel is suggested for further studies on wound healing.

REFERENCES

1. Potter PA, Perry AG, Stockert P, Hall A. Fundamentals of Nursing-E-Book. Elsevier Health Sciences; 2016 Feb 2.
2. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiological reviews. 2003 Jul;83(3):835-70.
3. Desmouliere, A., Redard, M., Darby, I., & Gabbiani, G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *The American journal of pathology*, 1995;*146*(1):56.
4. Dyson, M., Young, S., Pendle, C. L., Webster, D. F., & Lang, S. M. Comparison of the effects of moist and dry conditions on dermal repair. *Journal of investigative dermatology*, 1988;*91*(5):434-439.

5. Cohen K, Dieglemann RF, Yager Dr. Wound care and wound healing. In: Schwartz SI, Shires GT, Spencer FC, Daly JM, Fischer JE, Galloway AC(editors). Principles of surgery, companion handbook. New York: McGraw-Hill Professional, 1998;263-95.
6. Odimegwu, D. C., Ibezim, E. C., Esimone, C. O., Nworu, C. S., & Okoye, F. B. C. Wound healing and antibacterial activities of the extract of Dissotis theifolia (Melastomataceae) stem formulated in a simple ointment base. *Journal of Medicinal Plants Research*, 2008;*2*(1):011-016.
7. Lewis, W. H., & Elvin-Lewis, M. P. Medicinal plants as sources of new therapeutics. *Annals of the Missouri Botanical Garden*, 1995;16-24.
8. World Health Organization (WHO). *Traditional medicine growing needs and potential.* WHO Policy Pers. Med, 2002;2:1-6
9. Naqvi, S., Khan, M. S. Y., & Vohora, S. B. Anti-bacterial, anti-fungal and anthelmintic investigations on Indian medicinal plants. *Fitoterapia*, 1991;*62*:221-228.
10. Saxena, A., & Vikram, N. K. Role of selected Indian plants in management of type 2 diabetes: a review. *The Journal of Alternative & Complementary Medicine*, 2004;*10*(2):369-378.
11. Jurenka, J. Therapeutic applications of pomegranate (Punica granatum L.): a review. *Alternative medicine review*, 2008;*13*(2):128.
12. Riahy, S., Imany, H., & Khoshbaten, A. Effect of topical application of phenytoin and honey in closure of open wound in male rats. *HBI_Journals*, 2009;*7*(2):73-79.
13. McManus, J. F. A., & Mowry, R. W. Staining Methods, Histologic and Histochemical. Harper and Row, New York, Evanston, London, 1961.
14. Süntar, I., Akkol, E. K., Keleş, H., Oktem, A., Başer, K. H. C., & Yeşilada, E. A novel wound healing ointment: a formulation of Hypericum perforatum oil and sage and oregano essential oils based on traditional Turkish knowledge. *Journal of ethnopharmacology*, 2011;*134*(1): 89-96.
15. Allahtavakoli, M., & Khaksar, M. Assar Sh. *Comparison the effect of Mummify and Phenitoin ointment on skin wound healing. J Babol Univ Med sci*, 1993;*18*(5):7-13.
16. Blomhoff, R. Antioxidants and oxidative stress. *Tidsskrift for den Norske laegeforening: tidsskrift for praktisk medicin, ny raekke*, 2004;*124*(12):1643-1645.
17. Aliyev, E., Sakallıoğlu, U., Eren, Z., & Açıkgöz, G. The effect of polylactide membranes on the levels of reactive oxygen species in periodontal flaps during wound healing. *Biomaterials*, 2004;*25*(19):4633-4637.
18. Sönmez, M., Türk, G., & Yüce, A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology*, 2005;*63*(7):2063-2072.
19. Sönmez, M., Yüce, A., & Türk, G. The protective effects of melatonin and vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated male rats. *Reproductive Toxicology*, 2007;*23*(2):226-231.
20. Artik, N. Determination of phenolic compounds in pomegranate juice by using HPLC. *Fruit Processing*, 1998;*8*:492-499.
21. Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, Nitecki S, Hofman A, Rosenblat M, Volkova N, Presser D, Attias J. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs under experimental and clinical research. 2002;28(2-3):49-62.
22. Özkan, M., Kırca, A., & Cemeroğlu, B. Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. *Food chemistry*, 2004;*88*(4):591-597.
23. Lin, C. C., Hsu, Y. F., Lin, T. C., & Hsu, H. Y. Antioxidant and hepatoprotective effects of punicalagin and punicalin on acetaminophen-induced liver damage in rats. *Phytotherapy Research*, 2001;*15*(3):206-212.
24. Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., & Jiang, Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, 2003;*23*(12):1719-1726.
25. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food chemistry*, 2006;*96*(2):254-260.
26. Ambigaipalan, P., de Camargo, A. C., & Shahidi, F. Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. *Journal of agricultural and food chemistry*, 2016;*64*(34):6584-6604.
27. Abu-Al-Basal, M. A. Healing potential of Rosmarinus officinalis L. on full-thickness excision cutaneous wounds in alloxan-

induced-diabetic BALB/c mice. *Journal of ethnopharmacology*, 2010;*131*(2):443-450.
28. Heersaiy, A., & Farhadpour, M. R. Borago Officinalis Hydroethanolic Extract improved full thickness wound healing process in experimental animals. *International Journal of Biology, Pharmacy and Allied Sciences*, 2015;*4*(2):573-82.
29. Tümen, İ., Akkol, E. K., Taştan, H., Süntar, I., & Kurtca, M. Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (Pinus pinaster Ait). *Journal of ethnopharmacology*, 2018;*211*:235-246.
30. OZBİLGİN S, Akkol EK, ERGENE OZ B, Ilhan M, SALTAN G, BAHADIR ACIKARA O, Tekin M, Keleş H, SUNTAR İ. In vivo activity assessment of some Tanacetum species used as traditional wound healer along with identification of the phytochemical profile by a new validated HPLC method. Iranian Journal of Basic Medical Sciences. 2018 Jan 1.
31. Hsu, S. Green tea and the skin. *Journal of the American Academy of Dermatology*, 2005;*52*(6):1049-1059.
32. Klass, B. R., Branford, O. A., Grobbelaar, A. O., & Rolfe, K. J. The effect of epigallocatechin-3-gallate, a constituent of green tea, on transforming growth factor-β1–stimulated wound contraction. *Wound Repair and Regeneration*, 2010;*18*(1):80-88.
33. Prudente AS, Loddi AM, Duarte MR, Santos AR, Pochapski MT, Pizzolatti MG, Hayashi SS, Campos FR, Pontarolo R, Santos FA, Cabrini DA. Pre-clinical anti-inflammatory aspects of a cuisine and medicinal millennial herb: Malva sylvestris L. Food and chemical toxicology. 2013 Aug 1;58:324-31.
34. Beldon, P. Basic science of wound healing. *Surgery-Oxford International Edition*, 2010;*28*(9):409-412.
35. Ye H. Impact of Mindfulness-Based Stress Reduction (MBSR) on Students' Social Anxiety: A Randomized Controlled Trial. NeuroQuantology. 2017 Dec 21;15(4).
36. Zhang Z. Evaluating the Effectiveness of an Intervention Program to Regulate Cognitive Emotion of Patients with Type 2 Diabetes. NeuroQuantology. 2017 Dec 21;15(4).
37. Song W. Effects of a Training Program on Lifestyle Modification for Adolescents Identified with Overweight. NeuroQuantology. 2017 Dec 22;15(4).