

## THE WOUND HEALING ACTIVITY OF FLOWER EXTRACTS OF *PUNICA GRANATUM* AND *ACHILLEA KELLALENSIS* IN WISTAR RATS

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**Keywords:** *Punica granatum*, *Achillea kellalensis*, wound healing, rat

More than 80% of the world's population depend upon traditional medicines for various skin diseases (1). Recently, the traditional use of plants for wound healing has received attention by the scientific community (1, 2). Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3 % of modern drugs (3). Wound healing is a complex process characterized by homeostasis, re-epithelization, and granulation tissue formation and remodeling of the extracellular matrix (4). Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction are abundant in the scientific literature (5, 6). A survey of the ethnobotanical studies, carried out in Iran, indicated the use of several of plant species by the inhabitants of the area, especially by those habiting the rural areas, for wound healing purpose (7-9). *Punica granatum* Linn., known locally as "Golnar-e-farsi", is an important medicinal plant in Iran whose flowers are used as astringent, hemostatic, antibacterial, antifungal, antiviral and as a remedy for cut wound, bronchitis, diarrhea, digestive problems, man sex power reconstituent, dermal infected wounds and diabetes in *Unani* medicinal (Iranian Traditional Medicine) literature (7-9). This flower was also used for the treatment of injuries from falls and grey hair of young man in the traditional Chinese medicine (10). *Punica granatum* contains polyphenol compound named pomegranate, ellagic acid, 3,3',4'-tri-O-methylellagic acid, ethyl brevifolincarboxylate, urolic and maslinic acids, and daucosterol (11, 12).

*Achillea kellalensis* Bioss. & Hausskn. a well-known traditional herb used in tribal medicine of

Iran is locally known as "Golberrenjas or Bumadaran-e-Sabzekoh". The species of *Achillea* spp. have been used as a remedy for edema, burns, wounds, carminative, indigestion, skin infection, gastric ulcer, anti-bacterial, hemorrhage, dysmenorrhoea, enema and diarrhea (7-9). *Achillea kellalensis* monoterpenoids: camphor (34.0%), borneol (12.6%),  $\alpha$ -thujone, cineol, bornyl acetate and camphene (13).

### MATERIALS AND METHODS

#### Plant materials

The male abortive flowers of *Punica granatum* L. (Punicaceae), is a shrub or small tree and considered to be a native of Iran, and the flowers of *Achillea kellalensis* Bioss. & Hausskn. (Asteraceae) were collected on the slopes of the Zagros Mountains (1700–1800 m), District of Chaharmahal and Bakhtiari, Iran, during May – June 2007 and authenticated by Botany Laboratory, Researches Centre of Medicinal, Aromatic and Spice Plants, Islamic Azad University, Iran.

#### Preparation of the extract

Ethanol successive extract of *Punica granatum*, yield: 10% for flower, were prepared. Ethanolic extract of flower samples tested positive for polyphenols in flower (Wang). The aqueous extract of *Achillea kellalensis* filtered on Whatman paper and lyophilized a residue (yield: 5%) for flower, showed the presence of monoterpenoids.

#### Animals

Male Wistar rats (180-200 g) of 2-3 months of age were used. The animals were housed in standard

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environmental conditions of temperature ( $22 \pm 3^\circ\text{C}$ ), humidity ( $60 \pm 5\%$ ) and a 12-h light/dark cycle. During experimental time, rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

### Wound healing activity

Wound induction and evaluation extracts for properties wound healing before the beginning of the wound healing experiments, the dorsal skin of the Wistar rats were shaved. Animals were anesthetized with 1.5 mg/kg, *i.p.* of Ketamin and Xylazine. A full thickness of the excision wound (circular area about 150 mm<sup>2</sup> and 2 mm depth) was created along the markings using toothed forceps, a surgical blade and pointed scissors (14).

The animals were divided randomly into four groups of nine rats each. Group 1 was treated with nitrofurazone ointment and served as a reference standard; groups 2 and 3 were treated topically with the simple ointment prepared from extract of *Achillea kellalensis* and *Punica granatum* (200 mg/kg/day), respectively, and group 4 was treated topically with the simple ointment (Control). The percentage of wound closure was calculated as follows using the initial and final area drawn on glass slides during the experiments (15):

$$\% \text{ of wound closure} = (\text{wound area on day } 0 \times \text{wound area on day } n) / \text{wound area on day } 0 \times 100$$

where n is a number of days (6<sup>th</sup>, 9<sup>th</sup>, and 16<sup>th</sup> day).

During the wound healing period and at the presented time intervals, the wound area was traced manually and photographed. The wound area was calculated using Auto CAD RL 14 software. At days 6<sup>th</sup>, 9<sup>th</sup> and 16<sup>th</sup> the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The excision skin biopsies were fixed in 4% formaldehyde solution 48 h during the experimentation period.

### Statistical analysis

The relative wound area was statistically analyzed using one-way ANOVA by the program "SAS ver. 6.12 full" and comparison of the means of the wound areas at different days evaluated by Duncan's test at  $p < 0.05$  level.

## RESULTS AND DISCUSSION

The ethanolic extract of *Punica granatum* flowers and aqueous extract of *Achillea kellalensis* flowers showed significant wound healing activity

Table 1. Effect of the treatments on the evolution of wounds in rats after 6, 9 and 16 days of topical application

Treatment	Inflammation cells			Collagen fibers			Re-epithelization			Organization of the collagen			Necrosis			Fibrin			
	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	6 <sup>th</sup>	9 <sup>th</sup>	16 <sup>th</sup>	
Control	+++	++	++	-	-	+	-	-	+	-	-	+	+	++	++	+++	++	++	+
Simple ointment	+	+	-	+	++	+	+	+	+	++	+	+	+	+	+	+	+	+	-
Standard drug Nitrofurazone (0.2%)	+	+	-	+	++	+	+	+	+	++	+	+	+	+	+	+	+	+	-
<i>Achillea kellalensis</i> (10% extract + simple ointment)	+	+	+	+	++	+	+	+	+	++	+	+	+	+	+	+	+	+	-
<i>Punica granatum</i> (10% extract + simple ointment)	+	+	-	+	++	+++	+	+	++	++	++	++	++	++	++	++	++	++	-

+: slight, ++: moderate, +++: extensive, -: absent

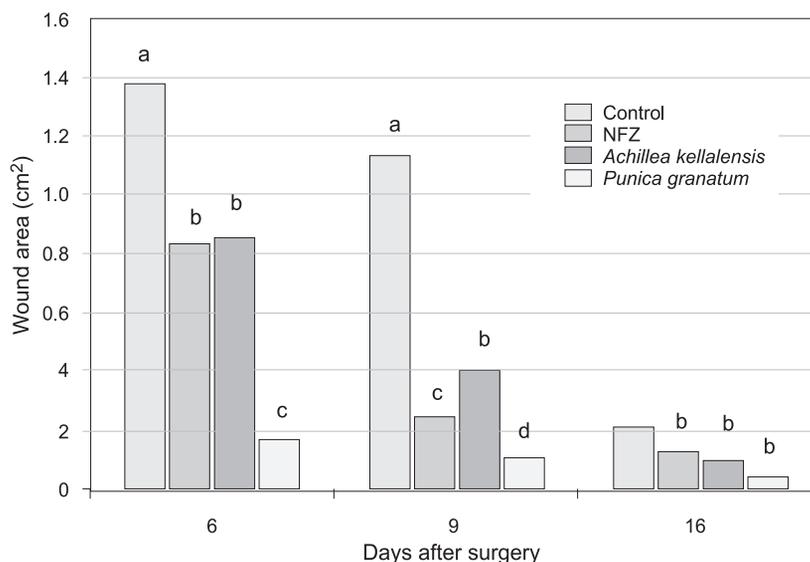


Figure 1. Comparison of means of the wound areas at different days of evolution by Duncan's test. The means with different letters are significantly different at 0.05 probability level according to Duncan's Multiple Range test

when topically administered in rats (Fig. 1.). The wound area measurement showed the wound size of the test groups were reduced early as compared to control group. The best results of histopathological evaluation were obtained with *Punica granatum*, when compared to the other groups as well as to the control and the standard drug (Table 1). These results offer pharmacological evidence on the folkloric use of *Punica granatum* flowers for healing wounds.

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. It is mainly dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair. In our study the extract of *Punica granatum* significantly increased the rate of wound contraction and collagen turnover. Collagen, the major component which strengthens and supports extracellular tissue, is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (16).

The preliminary phytochemical analysis of the flower extract of *Punica granatum* showed the absence of polyphenol compound named pomegranate; ellagic acid, 3,3',4'-tri-O-methylellagic acid, ethyl brevifolincarboxylate, urolic and maslinic acids, and daucosterol (10-12). Polyphenol compound may be responsible for antimicrobial activity.

It may be either due to the individual or additive effect of the phytoconstituents that hasten the process of wound healing. The exact component of the extract that is responsible for this effect, however, was not investigated. Further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

## CONCLUSIONS

The present study demonstrated that *Punica granatum* extract was capable of promoting wound healing activity. Enhanced wound contraction and histological observations suggest that *Punica granatum* has potential in the management of wound healing and suggests further study.

## Acknowledgments

The authors are thankful to Mr. F. Fadi Fard, Ph.D and Mr. M. Farid, Ph.D (Surgical) for their technical support.

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*Received: 25. 07. 2009*