



# An invivo Survey on Effect of Essential Oil of *Achillea millefolium* on Wound Healing in Chicken (Geometrical Study)

Amir Dehghani Samani<sup>1\*</sup>, Samin Madreseh Ghahfarokhi<sup>2</sup>, Azam Dehghani Samani<sup>2</sup>

<sup>1</sup>Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, 115, Iran

<sup>2</sup>Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, 115, Iran

## Abstract

Speed of healing is an important factor in wound healing. It is very important to find the natural drug that decrease this time. In current study effect of essential oil of *Achillea millefolium* on wound healing in 60 male chickens was study as a model for wound healing. Different concentration of essential oil of *Achillea millefolium* tested topically on full thickness excision wound with 25 mm diameter compared to phenytoin cream (1%) as a standard healing agent and geometrical study were done for 6 days. Result of this study show that higher concentrations (12% and 18%) have significant healing properties on chicken's wound. Treated groups with higher concentrations and also positive control group that treated with phenytoin cream have significant differences with negative controls and lower concentrations (1% and 6%). This study and same studies can help us to find the natural alternative components for wound healing without any side effects.

**Keywords:** *Achillea millefolium*, chicken, essential oil, wound healing

## 1. Introduction

Skin diseases occur in all countries and about 80% of the world's populations depend upon traditional medicines for various skin diseases [1]. Wound repair is a natural reaction to injury which results in restoration of tissue integrity. Repair process of wound will complete within 3 phases including: inflammation, proliferation and remodeling. There is a similarity between wound healing in human and certain animal species. Recently, the traditional use of plants for wound healing has received attention by the scientific community [1, 2].

The genus *Achillea* (Asteraceae), named after the mythological Greek warrior Achilles, who used *Achillea* species for healing wounded-soldiers during the Trojan

War [3]. The genus *Achillea* comprises of -85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 *Achillea* species and 23 of them are endemic [4]. These species have some interesting properties and are used in cosmetics, fragrances and agriculture, for example, plant protection [5]. Some *Achillea* species have been known to be ethnopharmacologically used in folk remedies for various purposes such as hemorrhoid and wound healing [6]. Herbal teas prepared from some *Achillea* species are very often used in folk medicine as diuretic, for abdominal pain, against diarrhea, flatulence and emmenagog, moreover for wound healing purposes [7–9]. Several biological activity studies have been performed on various *Achillea* species, including antibacterial, antioxidant, anti-inflammatory and antispasmodic activities [10–13].

\*Author for correspondence

E-mail: amirds2008@gmail.com

The aim of this study is show the effect of essential oil of *Achillea millefolium* on wound healing in chickens as a model for wound healing in humans and other animals.

## 2. Materials and Methods

### 2.1 Plant Material

A wild-growing plant from Kerman province's mountains located in southeast parts of Iran was collected in April and May, 2013. The samples, which were identified and confirmed as *Achillea millefolium*, were deposited in the Herbarium of the Research Institute of Jahade Keshavarzi of Shahrekord, Iran.

### 2.2 Isolation of the Volatile Oil

10 kg from aerial parts of the plant were dried in an oven equipped with warm air circulation. One hundred grams of the air-dried material was ground and powdered. The powder was subjected to hydro distillation for 3 h using a Clevenger-type system. Finally 30 ml essential oil extracted. The oil was kept at 2 to 4°C in a sealed brown vial.

### 2.3 Chickens

60 Adult (7 months old) roosters (Leghorn Breed) with 2000–2400 gram weight were studied. Chickens feed by standard pellet diet (Salehkashmar co, Iran); contain soybean meal and corn for 3 weeks. The chickens were housed in standard environmental conditions of temperature ( $25 \pm 3^\circ\text{C}$ ), humidity ( $55 \pm 5\%$ ) and a 16-h light/8-h dark cycle.

### 2.4 Wound Healing Studies

A full thickness wound was made in the skin of the test chickens according to the model of Cross [14] and the experiment was performed according to the modification of Hemmati and Mohammadian [15]. Feathers of lower back were fully picked and cleared, the desired area was locally anaesthisied with the subcutaneous injection of lidocaine (10%), the animal was held in standard crouching position, and the mobile skin of lower back was gently stretched and held by the fingers. A metal dick template measuring with 25 mm diameter was placed on the stretched skin and an outline of the template was traced on the skin using a finetipped pen. The wound was made by excising the skin, within the border of the

template to the level of loose subcutaneous tissue, using a size 15 scalpel blade and a forceps.

Wounds in positive control group were treated topically with 1 gram cream containing 1% phenytoin (Darupakhsh Co, Iran) as a standard healing agent twice a day (every 12 hours) and in tested groups treated with 5 ml solution contained various concentrations of essential oil; 1% (1 ml essential oil in 100 ml distilled sterile water), 6% (6 ml essential oil in 100 ml distilled sterile water), 12% (12 ml essential oil in 100 ml distilled sterile water) and 18% (18 ml essential oil in 100 ml distilled sterile water) twice a day (every 12 hours). Negative control group receive no treated.

All ethical issues were considered in the surgical procedure and during the treatments. Bedding in the cages was changed daily and cages were kept clean to avoid infection of wounds. Wound dressings were done twice daily.

Diameter of the wound for every chicken measured in days 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 at the same time in days. Measurement's errors were minimized by repeating each measurement in three times and using an average of the measurement in all calculations. Diameter size of the wounds was the base of comparisons and data analysis.

### 2.5 Statistical Analysis

Means were separated by one-way Analysis Of Variance (ANOVA) and significant differences ( $P < 0.05$ ) between means were assessed by Tukey's test using Sigma Plot software version 12.0.

## 3. Results

Diameter of the wound for every chicken significantly ( $P < 0.05$ ) reduced in day 1 compared day 0 but there were no significant difference between different test groups ( $P > 0.95$ ).

In day 2 diameter of the wound for groups 18% and positive control significantly ( $P < 0.05$ ) reduced compared day 1 and other tested groups.

Also in day 3 diameter of the wound for groups 18% and positive control significantly ( $P < 0.05$ ) reduced compared day 2, but there were no significant difference between other test groups ( $P > 0.95$ ) again.

In day 4 diameter of the wound for groups 18% and positive control significantly ( $P < 0.05$ ) reduced compared

day 3, but there were no significant difference between other test groups ( $P>0.05$ ), too.

In day 5 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 4.

Also in day 6 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 5.

In day 8 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 6, too.

Also in day 10 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 8, too.

In day 12 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 10 again.

And finally in day 14 diameter of the wound for each groups significantly ( $P<0.05$ ) reduced compared day 12.

Comparative data for each groups in every times show in Table 1.

## 4. Discussion

Wounds occur in every body's life, healing of wound is a complex phenomenon involving various phases e.g. coagulation, inflammation, collagenation, wound contraction and epithelization. While the phases between coagulation to collagenation are intimately interlinked,

the phases of wound contraction and epithelization are independent to each other and run concurrently [16].

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. When a wound occurs and is exposed to external environment, it is more prone to attack by microbes, which invade through the skin and delay the natural wound healing process. Reactive Oxygen Species (ROS); are vital part of healing and serve as cellular messengers that drive numerous aspects of molecular and cell biology. ROS can trigger the various beneficial pathways of wound healing, for example, at micromolar concentrations of hydrogen peroxide can promote Vascular Endothelial Growth Factor (VEGF) expression in keratinocytes [17–19]. During the inflammation phase of healing neutrophils and macrophages are attracted into the injured tissue by various chemotactic factors. They locate, identify, phagocytize, kill and digest microorganisms and eliminate wound debris through their characteristic “respiratory burst” activity and phagocytosis [20]. At high concentrations, ROS can induce severe tissue

**Table 1:** Mean size of wound's diameter (mm)  $\pm$  SEM in various test groups in different days

Groups Day	1%	6%	12%	18%	Positive Control	Negative Control
0	25 $\pm$ 0.00 a	25 $\pm$ 0.00 a	25 $\pm$ 0.00 a	25 $\pm$ 0.00 a	25 $\pm$ 0.00 a	25 $\pm$ 0.00 a
1	21.92 $\pm$ 0.14 b	21.66 $\pm$ 0.26 b	20.84 $\pm$ 0.08 b	20.96 $\pm$ 0.94 b	20.01 $\pm$ 0.63 b	21.98 $\pm$ 1.07 b
2	19.34 $\pm$ 1.13 bd	18.08 $\pm$ 0.94 bd	18.65 $\pm$ 0.24 bd	16.52 $\pm$ 1.31 bc	15.28 $\pm$ 1.65 bc	20.29 $\pm$ 0.37 b
3	18.13 $\pm$ 0.29 bd	16.08 $\pm$ 0.85 bd	17.26 $\pm$ 1.06 bd	13.86 $\pm$ 0.44 be	13.14 $\pm$ 0.09 be	18.28 $\pm$ 0.67 bd
4	16.24 $\pm$ 1.63 bd	15.45 $\pm$ 0.92 bd	14.69 $\pm$ 0.66 bd	12.52 $\pm$ 0.77 bf	11.36 $\pm$ 0.28 bf	16.13 $\pm$ 0.71 bd
5	13.56 $\pm$ 0.06 bh	13.74 $\pm$ 1.49 bh	12.07 $\pm$ 0.09 bi	10.62 $\pm$ 0.70 bj	9.99 $\pm$ 0.51 bj	13.89 $\pm$ 1.83 bh
6	11.02 $\pm$ 0.94 bk	10.27 $\pm$ 0.45 bk	9.24 $\pm$ 0.17 bl	7.85 $\pm$ 0.96 bm	7.12 $\pm$ 2.07 bm	11.05 $\pm$ 0.53 bk
8	9.32 $\pm$ 0.05 bn	8.88 $\pm$ 2.42 bn	6.98 $\pm$ 1.87 bo	5.83 $\pm$ 0.76 bp	5.14 $\pm$ 0.71 bp	8.75 $\pm$ 0.69 bn
10	7.46 $\pm$ 1.83 bq	7.01 $\pm$ 0.29 bq	5.47 $\pm$ 0.04 br	4.99 $\pm$ 2.34 br	4.56 $\pm$ 0.71 br	8.13 $\pm$ 1.21 bq
12	6.36 $\pm$ 0.99 bq	5.95 $\pm$ 0.66 bs	5.04 $\pm$ 0.66 bs	4.11 $\pm$ 0.01 bt	3.89 $\pm$ 1.07 bt	7.58 $\pm$ 0.57 bq
14	5.62 $\pm$ 1.18 bs	5.09 $\pm$ 0.15 bu	4.35 $\pm$ 2.28 bu	3.84 $\pm$ 0.81 bv	3.25 $\pm$ 0.13 bv	6.84 $\pm$ 2.36 bq

Different lower cases in rows show the significant difference between every tested group and different lower cases in columns show the significant difference between every day.

damage and even lead to neoplastic transformation, which further impede the healing process by causing damage to cellular membranes, DNA, proteins and lipids as well [21]. Hence, if a compound or a plant extract having antioxidant potentials and antimicrobial activity additionally, it can be a good therapeutic agent for accelerating the wound-healing process.

*Achillea* species have been so far reported to contain diterpenes, sesquiterpenes, flavonoids, lignans, essential oil and rarely triterpenes [22, 23]. Various biological activity studies were also completed on *Achillea* species. The antimicrobial and antioxidant activities of the essential oil and the methanolic extract of *A. biebersteinii* were studied in vitro by [24]. The essential oil showed antimicrobial activity against 8 bacteria sp., 14 fungi sp. and the *Candida albicans*, whereas the methanolic extract remained inactive. Several preparations containing *A. millefolium* extract was quite successfully healed the wounds and scars. The liniment containing hiperisin oil and *A. millefolium* extract patented by *Motogna* accelerates the healing of wounds and gives esthetic scars. Since the liniment is applied as a spray it is easily applied and painless [25].

In current study effect of essential oil of *Achillea millefolium* on wound healing in chickens studied as a model for wound healing. Results obtained from this study show that essential oil of *Achillea millefolium* in highest concentrations (12 and 18%) have promoting wound healing activity that we can use from this essential oil in wounds without any side effects.

The present study demonstrated that essential oil of *Achillea millefolium* was capable of promoting wound healing activity. So we advised that effect of this essential oil on wound healing in other animals and also in humans study in future work. Also effect of this essential oil on wound healing in gastro intestinal tubes can be study in same works.

## 5. Acknowledgement

We take this opportunity to express a deep sense of gratitude to all of them who helped us in this research work.

## References

1. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of

- Ficus asperifolia* Miq. and *Gossypium arboreum* L., wound-healing plants of Ghana. J Ethnopharmacol. 2008; 119:141–44.
2. Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. In vitro tests and ethnopharmacological investigations: wound healing as an example. J Ethnopharmacol. 2005; 100:100–7.
3. Cheers G. Botanica. Koln, Germany: Konemann; 1999.
4. Duman H. Achillea. In: Guner A, Ozhatay N, Ekim T, Baser KHC, editors. Flora of Turkey and the East Aegean Islands. Edinburgh, UK: Edinburgh University Press; 2000; 11:158–9.
5. Senatore F, Napolitano F, Arnold NA, Bruno M, Herz W. Composition and antimicrobial activity of the essential oil of *Achillea falcata* L. (Asteraceae). Flavour Fragrance J. 2005; 20(3):291–4.
6. Baytop T. Therapy with medicinal plants in Turkey, past and present. 2nd ed. Istanbul, Turkey: Nobel Tip Kitapevi; 1999.
7. Fujita T, Sezic E, Tabata M, Yesilada E, Honda G. Traditional medicine in Turkey VII. Folk medicine in middle and west Black Sea regions. Econ Bot. 1995; 49(4):406–22.
8. Honda G, Yesilada E, Tabata M, Sezic E, Fujita T, Takeda Y et al. Traditional medicine in Turkey VI. Folkmedicine in West Anatolia: Afyon, Kutahya, Denizli, Mugla, Aydin provinces. J Ethnopharmacol. 1996; 53(2):75–87.
9. Yesilada E, Honda G, Sezic E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in turkey IV. Folk medicine in the Mediterranean subdivision. J Ethnopharmacol. 1993; 39(1):31–8.
10. Karamenderes C, Apaydin S. Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bassler on the rat isolated duodenum. J Ethnopharmacol. 2003; 84(2–3):175–9.
11. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A et al.. Antioxidant and antimicrobial activity of the essential oils and methanol extracts of *Achillea millefolium* susp. *millefolium* Afan. (Asteraceae). J Ethnopharmacol. 2003; 87:215–20.
12. Al-Hindawi MK, Al-Deen IH, Nabi MH, Ismail MA. Anti-inflammatory activity of some Iraqi plants using intact rats. J Ethnopharmacol. 1989; 26(2):163–8.
13. Skocibusic M, Bezic N, Dunkic V, Radonic A. Antibacterial activity of *Achillea clavennae* essential oil against respiratory tract pathogens. Fitoterapia. 2004; 75(7–8): 733–6.
14. Cross SE, Naylor IL, Coleman RA, Teo TC. Modification to the rate of wound contraction by allopurinol. Br J Plastic Surgery. 1995; 48:189–97.

15. Hemmati AA, Moahammadian F. An investigation into the effect of mucilage of quince seeds on wound healing in rabbit. *J Herbs Spices Med Plants*. 2000; 7:41–46.
16. Champion RH, Burton JL, Burns DA, Breathnach SM. *Textbook of dermatology*. Oxford, Blackwell Science; 1998.
17. Khanna S, Roy S, Bagchi D, Bagchi M, Sen CK. Upregulation of oxidant-induced VEGF expression in cultured keratinocytes by a grape seed proanthocyanidin extract. *Free Rad Biol Med*. 2001; 31(1):38–42.
18. Prasad AS, Oberleas D. Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissue. *J Lab Clin Med*. 1974; 83:634.
19. Nayak BS, Sandiford S, Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia*. *Altern Med*. 2009; 6(3):351–6.
20. Clark LA, Moon RE. Hyperbaric oxygen in the treatment of life-threatening soft-tissue infections. *Respir Care Clin No Am*. 1999; 5(2):203–19.
21. Martin A. The use of antioxidants in healing. *Dermatol Surg*. 1996; 22(2):156–60.
22. Ahmed AA, Mahmoud AA, Ali ET, Tzakou O, Couladis M, Mabry TJ et al. Two highly oxygenated eudesmanes and 10 lignans from *Achillea holosericea*. *Phytochemistry*. 2002; 59(8):851–6.
23. Mockute D, Judzentiene A. Variability of the essential oils composition of *Achillea millefolium* ssp. *millefolium* growing wild in Lithuania. *Biochem Syst Ecol*. 2003. 31(9):1033–45.
24. Baris O, Gulluce M, Sahin F, Sengul M. Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan (Asteraceae). *Turk J Biol*. 2006; 30(2):65–73.
25. Motogna A. Liniment for treating serious burns. (Combinatul de Industrie Locala), Rom. RUXXA3, RO 52874, 19711125, Appl., RO 68-58261; 1971.