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# Evaluation of the burn healing properties of oil extraction from housefly larva in mice

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ABSTRACT

*Aim of the study:* To evaluate the burn wound-healing properties of oil extraction from housefly larva by employing a deep second-degree model in mice.
*Materials and methods:* The burn wound-healing properties were evaluated by employing a deep second-degree model of burn wound assay. The anti-inflammatory activity and antibacterial activity were examined by employing the xylene-induced ear swelling model in mice and a disc-diffusion assay, respectively.
*Results:* The best wound-healing activity was observed with the oil extract of housefly larva, followed by Ching Wan Hung® and the least active was that of saline solution. The oil extraction from housefly larva suppressed xylene-induced ear swelling in mice with 35.3% of the inhibition rate at the dose of 0.1 ml/30 g. The disc-diffusion assay of housefly larva oil showed positive effect to *Pseudomonas aeruginosa* and *Staphylococcus aureus*, exhibiting no antibacterial activity to *Escherichia coli*.
*Conclusion:* The study provides useful information that the housefly larva oil could be used as a natural ointment to heal the burn wound.


## 1. Introduction

Insects are a large, unexplored and unexploited source of potentially useful compounds for modern medicine (Pemberton, 1999). A great array of insects and their products are used as drugs in the traditional medicines such as traditional Chinese and Korean medicine, but few researches in this area have been reported. The housefly (*Musca domestica)* belongings to the Diptera and the larva of housefly *Musca domestica* Linnaeus (Diptera: Muscidae) is an excellent source of high-quality protein, polyunsaturated fats, vitamins, minerals and other nutrients for human and animal (Ren and Shi, 2002). The housefly larva has been used clinically to cure skin scald, malnutritional stagnation, decubital necrosis, osteomyelitis, ecthyma and lip boil since the 14th century in China, and also used to treat coma and gastric cancer when combined with other drugs (Jiang, 1999).

Despite it has also been used in medicine and functional food for centuries, only few reports are available on the bioactive constituents from the larva of housefly. The objective of this study was to evaluate the burn wound-healing properties of oil extraction from housefly larva in mice. In the present study, the composition of fatty acid, a deep second-degree model of burn wound assay, xylene-induced ear swelling model in mice, and a disc-diffusion were also examined. Histological evaluation of the skin of the mice was also evaluated.

## 2. Materials and methods

### 2.1. Preparation of housefly larva oil

Housefly larva was provided by the Institute of Insect Resources, Huazhong Agricultural University, Wuhan, Hubei. The Fourth-instar larva of housefly M. domestica was collected, the insect materials were identified by Dr. Chao-Liang Lei at the Institute of Insect Resources, Huazhong Agricultural, and washed by distill water, frozen and lyophilized. The dried larva was ground using a mill to obtain crude power. The lyophilized was extracted with petroleum ether (boiling process 30–60 °C) in an ultrasonic apparatus for 3 times, 20 min each time at room temperature. The extractions were collected together and concentrated in a vacuum at 50 °C by a rotary evaporator. Then the oil was evaporated by a vacuum dryer until it reached a constant weight (Wang et al., 2007).

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### 2.2. Gas chromatography analysis of the housefly larva oil

The gas chromatography analyses were accomplished using a Agilent 6890 Series gas chromatograph equipped with a FID and DB-23 capillary columns (30 m × 0.25 mm, i.d. × 0.25 μm) working with the following temperature program: 120 °C for 4 min, rising at 10 °C/min to 250 °C, holding for 20 min, injector and detector's temperature 250 °C and 270 °C, respectively. Carrier gas was nitrogen (30 ml/min). The percentage composition was obtained from electronic integration measurements using flame ionization detection (Ozturk and Ercisli, 2006).

### 2.3. Evaluation of the burn wound-healing properties

#### 2.3.1. Animals

All animals received humane care and the studies reported in this manuscript have been carried out in accordance with the guidelines for the humane treatment of animals set by the Association of Laboratory Animal Sciences at College of Life Sciences, South Central University for Nationalities. Sixty SPF-class Kunming rats (30.0 ± 5.0 g, 2- to 3-month-old, male and female is fifty–fifty) were obtained from the Animal Center of Hubei Provincial Center for Diseases Control and Prevention, and kept for at least 1 week on a commercial solid diet under controlling conditions (25 ± 2 °C, 12 h light/dark cycle, 55 ± 5% humidity) with free access to food and water before treatment (Wang et al., 2007).

#### 2.3.2. Burn experiment

*2.3.2.1. Burn injury.* In the beginning, the dorsal skins of the mice were depilated by 2% CMC-Na and 10% sodium sulfide solution. 24 h, under chloral-hydrate anesthesia (0.1 ml 10% chloral-hydrate solution was administered intraperitoneal injection). The dorsal skin of the mice was immersed into the water of 85 °C for 15 s. Only the animals, which created a deep second-degree model of burn and the burn area was approximately 10% of the animal's total body surface area (approximately 2.5 cm × 2.5 cm), were chosen. The process was affirmed by a pathologist (Khalil et al., 2007) and four mice were eliminated for they could not accord with the criteria of a deep second model of burn (Feng et al., 2006). After wound creation, 0.1 ml 0.85% saline solution was administered intraperitoneal injection immediately.

*2.3.2.2. Experimental protocols.* After wound creation the mice were divided randomly into three groups. Group one was treated immediately with the test oil (housefly larva oil), group two was with Ching Wan Hung®, 0.3 ml/100 g per mice, and group three was treated with 0.3 ml/100 g saline solution per mice as control group. All the reagents were applied slowly on the wound area, extending slightly outside the wound area to ensure inclusion of the wound edges. The wounds were treated with the different preparations with 24 h intervals for 30 days. The first application was done directly after the wound injuring (Khalil et al., 2007).

This model was used to monitor wound contraction, wound closure time and the edema, exudation and the firm of the wound surface was also observed. Wound contraction was calculated by percent reduction in wound size. Optical photographs were taken from the wound area, using a professional Canon PowerShot A510 camera at an equal distance from the wound and right angle to the wound surface at 5 days intervals (Khalil et al., 2007). The progressive changes in the wound size were monitored by printing these photographs and tracing the wound margin on the weighting paper. The wound size was expressed by the mass of the weighting paper (Saha et al., 1997).

$$\text{The decrease of wound size } (\%) = \frac{\text{wound size day } 0 - \text{wound size day } X}{\text{wound size day } 0} \times 100\%$$

The wound closure time was expressed as mean and standard deviation. During the experimentation periods, the wound surface was observed from these criteria: the edema, exudation and the firm of the wound surface. Different animal groups were assessed blindly by the pathologist and the results were compared with the control group (Khalil et al., 2007).

At day 30 the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The excisional skin biopsies were fixed in 4% neutral buffered formaldehyde solution for 24 h. Cross-sections through the longitudinal aspect of the scarred areas were made. The tissue was processed in the routine way for histological evaluation and embedded in paraffin, staining with hematoxylin and eosin, the routine stain used in the histopathology, and recommended as a general survey stain. Tissue samples were evaluated for the following histological criteria: the extent of re-epithelization, the maturation and organization of the epidermal squamous cells, the thickness of the granular cell layer, the degree of granulation tissue formation, the boundary between the epidermal and the dermis, the structure of the hair follicles. The different animal groups were assessed blindly by the pathologist and the results were compared with the control group (Khalil et al., 2007).

#### 2.3.3. Anti-inflammatory activity test

The mice were divided randomly into three groups, consisting of ten mice per group. The anti-inflammatory activity tested including housefly larva oil, Ching Wan Hung® and saline solution (0.1 ml/30 g) was spread on the anterior and posterior surfaces of the ears lobe.

One day later, each animal received 0.1 ml of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was considered as control. After 15 min, the animals were killed by cervical dislocation and both ears were sampled. Circular sections were taken, using a cork borer with a diameter of 6 mm, and weighed. The degree of ear swelling was calculated based on the weight of left ear without applying xylene (Zhou et al., 2008).

#### 2.3.4. Antibacterial activity test

The disc-diffusion assay was used to determine the growth inhibition caused by oil extracts against the following bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*. These bacteria, maintained at 4 °C on nutrient agar (NA) plates, were supplied by Food Microbiology Laboratory of College of Food Science and Technology of Huazhong Agricultural University, Wuhan, China.

Plates were prepared by pouring 10 ml nutrient agar (NA) into sterile Petri dishes (9 cm) and allowed to set. Nutrient agar held at 37 °C was inoculated with 1 ml $10^2$–$10^3$ bacteria per milliliter) of the test organism and poured over the base plates forming a homogenous top layer. Filter paper discs (Double Circle No. 102, 6 mm diameter) were sterilized by autoclaving. The filter paper discs were emerged into the tubes which contained the housefly larva oil for 40 min to form solvent saturated discs. Then the discs were air-dried and placed onto the surface of the nutrient agar plates. The housefly larva oil was tested in quadruplicate (four discs per plate); with an air-dried saline solution saturated discs were used as control. The plates were evaluated after incubation at 37 °C for 24 h after which the zones of inhibition around each disc were measured. The inhibition zone (mm) produced by the housefly larva oil was used to express antibacterial activity (Matu and Van Staden, 2003).

### 2.4. Statistical analysis

All the results are expressed as mean and standard deviation. Results between treatments were compared using one-way analy-