

# Growth Control of *Acinetobacter baumannii* in Infected Wound by Oregano Essential Oil in Rats

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## ABSTRACT

**Introduction:** *Acinetobacter baumannii* known as a main agent in nosocomial infections and its drug resistance is a medical problem in hospitals. In the present study, growth control of *Acinetobacter* by Oregano essential oil was investigated in-vitro and in experimental wounds on rats.

**Materials and Methods:** Oregano essential oil (OES) was prepared by a Clevenger apparatus in a hydrodistillation process. Susceptibility of clinical isolate of *Acinetobacter* to essential oil was evaluated by disk diffusion and microbroth dilution methods. Ketamine and xylazine were used for surgical anesthesia induction in rats. The backs of 30 rats were shaved and an experimental wound in one square inch was prepared between two scapulae. All the ulcers were infected with fresh culture of *Acinetobacter*. The rats were divided into three equal groups for treatment by OES twice a day, treatment with silversulfuridene ointment (SSO) and control group without any treatment. Infection symptoms were assessed until the wound was fully granulated.

**Results:** MIC and MBC were as 4 mg/ml and 6 mg/ml, respectively. Blood culture of control group and SSO contained *A. baumannii* but was negative in treatment group. The cultivation and examination of the wounds and its secretions revealed the absence of *A. baumannii* in treatment group. Wound healing was faster in the treatment group than in the control groups.

**Conclusion:** Infection of the wounds infected with *A. baumannii* was interestingly controlled by OES and subsequently accelerated the wound healing process in Wistar rats. Therefore, OES recommends for evaluation in a clinical trial.

**Key words:** Infected wound, *Acinetobacter baumannii*, Oregano essential oil, Rat

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## INTRODUCTION

*Acinetobacter baumannii* causes a wide range of infections, including urinary tract infections, meningitis, bacteremia, wound infections, skin and soft tissue infections, and respiratory infections, especially in hospital care units [1-4].

This bacterium is highly resistant to antimicrobial agents, and this resistance can be inherent or acquisitive. This bacterium has the ability to acquired resistance to various antibiotic classes, resulting in its multi-drug resistance. Today, *A. baumannii* shows resistance to most antibiotics including aminoglycosides, carbapenems, cephalosporins

and beta-lactams [3,5,6]. Multidrug-resistant (MDR) *A. baumannii* was detected in many hospitals on immunosuppressed patients [7]. Therefore, it is necessary to work on the antibacterial activity of traditional medicinal plants.

The use of plant compounds to control infections by this bacterium has attracted the attention of many researchers. Oregano is rich in essential oils with the main components of alpha thujene, alpha-pinene, octanone, terpinene, p-cymene, carvacrol and thymol. These compounds have antioxidant, anti-microbial, and antiproliferative properties in tumor cells, antithrombin and anti-hyperglycemia. Studies have been shown that this plant is useful in the treatment of cancer, heart disease and hypertension [8,9].

High antimicrobial capacity of Oregano essential oil have been proven against a wide range of bacteria including

different species of *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Escherichia coli*, as well as some fungal species such as *Aspergillus*, *Fusarium* and *Penicillium* [10].

A wound that does not heal after 48 hours is likely to be infected. The wound infection rate in clean and uncontaminated wounds is about 14% but contamination by drug resistant strains (e.g. *A. baumannii*) is occurred as a nosocomial infection in some hospitals [1,2,11].

We hypothesized that the Oregano Essential Oil has anti-*A. baumannii* effect. The purpose of this study was to *Acinetobacter* control *in-vitro* and in experimental wounds in Wistar rats.

## MATERIALS AND METHODS

### Bacterial strain

Clinical isolate of *Acinetobacter baumannii* strain was obtained from the microbial bank of the Infectious Disease Research Center of Arak University of Medical Sciences, Arak, Iran.

### Disc diffusion

The susceptibility of bacteria to Oregano essential oil was investigated. Fresh bacterial suspension in a concentration of  $1.5 \times 10^8$  CFU/ml was spread over a Mueller Hinton Agar medium surface. Blank disks were placed on the medium loaded with 20  $\mu$ L of diluted Oregano essential oil in various concentrations. The plates were placed in a 37°C incubator for 24 hours and the diameter of the zone of inhibition was measured at the end of the incubation period.

### Microplate dilution method

In order to determine the minimum inhibitory concentration (MIC), fresh culture of the isolate was added to 100  $\mu$ l of the prepared suspension in each of the 96-well plates. Then 100  $\mu$ l of diluted essential oil was added to the first well and repeated to well number 9 and finally discarded 100  $\mu$ l of the solution. In tenth well the bacteria suspension was poured as positive control. After 18-20 hours of incubation, the turbidity was measured using a spectrophotometer at a wavelength of 550 nm.

### Preparation of the plant and essential oil

The Oregano plant was washed twice and dried in a dark room in a  $23^\circ\text{C} \pm 2^\circ\text{C}$ . Afterwards, the dried plant was powdered by a chopper. 40 g of powdered plant plus 400 L water were placed into a glass balloon of a Clevenger and the essential oil was extracted using the heater.

### Animal study

Thirty rats were exposed to general anesthesia with ketamine and Xylazil and then their backs' hairs were shaved. 2.5 cm<sup>2</sup> wound was created between the rats' shoulders with a special surgical instrument.

### Wound infection

Fresh *Acinetobacter* suspension was inoculated using insulin syringes directly into the newly created wounds in all rats. Infection of the wounds was assessed by microscopic examinations and microbial culture in the Mueller Hinton Agar culture medium after 24 h and 48 h.

### Study groups

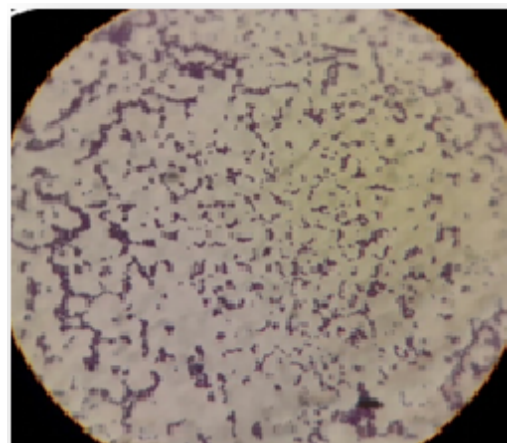
Wounded rats were divided into three groups of 10 rats. The treatment group was considered to be treated with essential oil, the control group with 1% Silver Sulfadiazine ointment, and the third group was without treatment and considered as control. The debridement process and the clinical signs of infection were examined until the wound was fully granulated.

Essential oil was sprayed on the wound with insulin syringe twice a day. This process continued until the end of the treatment period. The dose and volume of drug was determined following the microplate dilution test results.

## RESULTS

*Acinetobacter baumannii* was isolated from the blood culture of the control group and was sub-cultured into the Mueller Hinton Agar medium but any were separated from the blood culture of the treatment group.

Gram stain and culture of wound secretions revealed that gram-negative bacteria had grown on the culture medium and gram-negative bacterium had also grown in the culture of the wound, and the grown bacteria were identified as *Acinetobacter baumannii* (Figure 1).



**Figure 1:** The image of gram staining from the wound secretions of control group

Moreover, gram staining was prepared from wound secretions at the beginning and end of the treatment during the treatment period of the wounds, which confirmed the presence of a large community of *Acinetobacter baumannii* in the wounds at the start of treatment and the lack of this bacterium at the end of the treatment (Figure 2 and Figure 3).

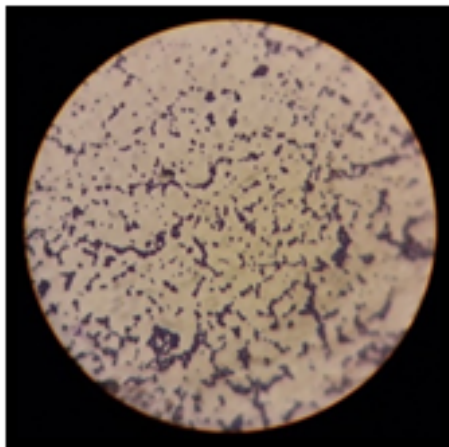


Figure 2: The presence of *Acinetobacter baumannii* in the rats' wound secretions at the beginning of treatment

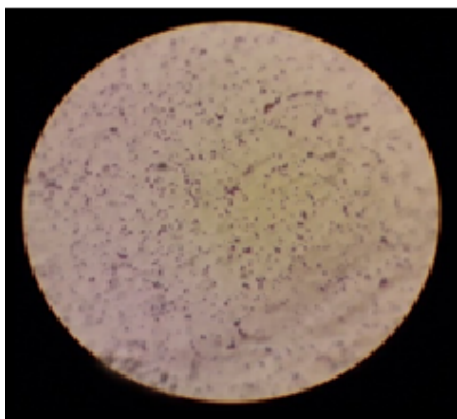


Figure 3: Lack of *Acinetobacter baumannii* in the rats' wound secretions at the end of treatment

In addition, the wound healing process that had been greatly improved by the effect of Oregano essential oil was proven (Figure 4).

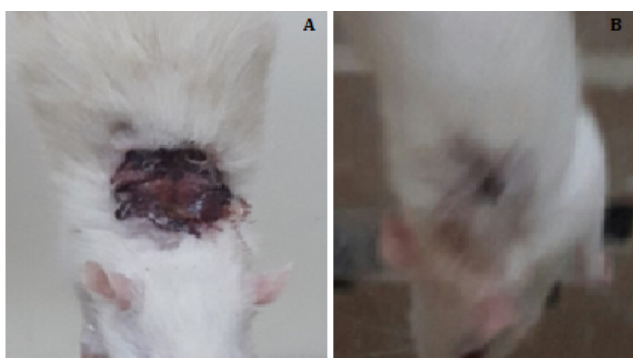


Figure 4: Healing effect of oregano essential oil: (A) Before herbal treatment; (B) After herbal treatment

### DISCUSSION

One of the major concerns in the treatment of infections caused by antibiotic-resistant bacteria is the high cost and long duration of the treatment.

The results of this study showed that Oregano essential oil was useful for the treatment of infected wounds by

*Acinetobacter baumannii* and also accelerates the wound healing process.

The healing process follows the natural trend of improvement in the case of acute wounds, while the healing process slows down or is disrupted in the case of chronic or non-healing wounds due to the involvement of an internal or external agent at one of the stages of recovery. Upon the creation of a wound, the body has to start and continue several responses simultaneously, including preventing the bleeding, preventing the attacks of bacteria and microorganisms, and removing the dead tissues and external objects from the wound site and producing new tissues in the wound.

The wound healing in the case of infected wounds is more complicated and depends on the amount of tissue damage and the type of infection. A simple accumulation of purulent substances inside the skin and subcutaneous tissue is treated through a surgical incision. If the wound infection happens to penetrate into the deeper parts of the skin or if there is a dangerous microbial agent that causes the subcutaneous tissue to be destroyed, it is necessary that a surgeon open the wound and remove the destroyed tissue and administer long-term antibiotics.

According to the previously conducted studies, Oregano essential oil can cure and shorten the healing duration of infectious wounds. Scandorieiro et al., from the University of Aichi Gakui conducted a study on the synergistic effect of Oregano essential oil and silver nanoparticles on several drug-resistant bacteria, including *Acinetobacter baumannii* bacteria; coming to the conclusion that essential oil in low concentration levels has bactericidal effect on all drug-resistant bacteria including *Acinetobacter baumannii* [12].

A study conducted in Iran by Pajohi et al. to evaluate chemical compounds and antimicrobial activity of oregano essential oils and cumin seed, and showed that Oregano essential oil has an appropriate antimicrobial effect [13].

The results of this study proved the effectiveness of Oregano essential oil without silver nanoparticles.

Several studies have dealt with the effects of other plants on *Acinetobacter*. One of these studies was conducted in Thailand by Phatthalung et al., who showed that the Thai herbal extracts such as black helium, when combined with Novobiocin, can positively affect *Acinetobacter baumannii* [14]. Another study was carried out by Sakkas et al. at Ioannina University of Greece on the antimicrobial activity of the essential oils of five plants on the Gram-negative drug-resistant bacteria, including *Acinetobacter baumannii*. This study showed that the essential oils of basil, marigold, tea tree and thyme can inhibit the growth of *Acinetobacter baumannii*, while chamomile has no antibacterial effect [15].

In the present study was proved the effect of Oregano essential oil on *Acinetobacter*.

**CONCLUSION**

The findings of this study indicate that Oregano essential oil can have significant antibacterial effects on *Acinetobacter baumannii*-infected wounds and subsequently accelerate the healing of infectious wounds in Wistar rats. Therefore, this plant is recommended for conducting clinical trials.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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