INTRODUCTION

Hydatidosis caused by *Echinococcus* spp. is a major zoonotic infection that is detrimental to both humans and animal husbandries in many countries. Cystic echinococcosis affects mainly the intermediate host’s viscera, including the liver, lungs, and less frequently, the spleen, kidneys, bone, brain, and other organs [1]. Currently the basic approaches for treatment of hydatid disease are surgery and chemotherapy. However, operative leakage may lead to dissemination of viable protoscolices to adjacent tissues and thus to intrapritoneal hydatid disease [2,3].

The olive (*Olea europaea*) tree, a plant which can survive for hundreds of years, is known to naturally possess strong resistance to microbial attack [4]. Natural olive leaf and olive leaf extracts are now marketed as anti-aging agents, immunostimulants, and even antibiotics. Olive leaf extracts have been used throughout history for their medical properties, for instance, treatment of infections. Several studies have shown a decreased risk of bacterial and parasitic protozoan diseases with an increasing consumption of olive products [4,5]. Although antiprotozoal activities of the competent Oleuropein (*O. europaea*) have been examined, anthelmintic potential of olive leaf extracts have not been reported.

*Satureja khuzestanica* Jamzad is an endemic plant that is widely distributed in the southern part of Iran. It is famous for its medical uses as an analgesic and antiseptic in folk medicine [6]. Recently, antiviral, antibacterial, antifungal, and antiprotozoal effects were investigated from various species of *Satureja* [7-15].

Therefore, the aim of this investigation was to examine the activity of Iranian medical plants, including *O. europaea* and *S. khuzestanica*, against protoscolices of hydatid cysts and to determine the exposure time and concentrations of the extracts providing scolicidal activities.

MATERIALS AND METHODS

Plant material

Leaves of *O. europaea* and the aerial parts of *S. khuzestanica* were collected in June 2010 from Khorram Abad (center of Lorestan province) in southwestern Iran, and identified by Botanical Section, Lorestan University of Medical Sciences. The
plants were dried in open air and shady conditions until completely dried and then ground to a powder. All experiments were performed with 1 batch of olive leaf and S. khuzestanica extracts, separately.

Preparation of aqueous plant extracts
A 200 g based on dry weight powdered leaves added to adequate amount water (1,000 ml) to concentration of 20% (w/v). The products were squeezed through gauze cloth to remove the practice and the extract was passed through a 0.2 µm filter (Millipore™ Membrane Filter, USA). The procedure of extraction and filtration were operated at room temperature. The extract was stored at 4˚C until use.

Preparation of hydroethanolic plant extract
S. khuzestanica extracts were prepared according to the method of Zarrin et al. [16]. Briefly, about 10 g powdered leaves of S. khuzestanica was extracted with adding 100 ml of 80% ethanol (1:10 w/v). After 72 hr at room temperature, the suspension was filtered through a Whatman paper No.1 and the crude ethanol extracts were evaporated at 37˚C. One gram of extract was dissolved in 1 ml of 100% dimethylsulfoxide (DMSO), and the final concentration of each extract was adjusted to 1,000 mg/ml.

Collection of protoscolices
Sixty hydatid cysts were collected from the liver of infected sheep slaughtered at Khorram Abad and carried to the Parasitology Laboratory at the Department of Parasitology and Mycology, Lorestan University of Medical Sciences. The protoscolices were obtained from the hydatid fluid and washed 3 times in PBS (pH 7.2). The concentration of protoscolices was confirmed as the number of protoscolices per ml of the hydatid fluid in a saline solution (0.9% NaCl solution) containing 5 × 10⁵ protoscolices in 1 ml with more than 90% viability was used in further use [2].

Viability assay
A 0.1 mg of eosin stain was dissolved in 100 ml distilled water at a 0.1% (w/v) concentration. The viability was checked microscopically after adding 10 µl of eosin solution to 10 µl of protoscolices for 15 min. Stained protoscolices were considered as dead while unstained protoscolices were recorded as alive. Non-treated protoscolices (with plant extracts) were considered as the control group.

Statistical analysis
The statistical analysis was performed with the use of SPSS version 15.0.1. The protoscolicidal activity was calculated as means ± SD.

RESULTS
The mortality rates of protoscolices of hydatid cysts after exposure to different concentrations of O. europaea extracts in various time periods are demonstrated in Table 1. Olive leaf extracts 0.1% had strong scolicidal effects in 120 min, and 0.01% also revealed the same effects at the same time. A 96.7% of protoscolices lost viability at 120 min (0.01% diluted). The mortality rate at 0.001% decreased to 53.1% at 30 min, while many of the protoscolices died at 0.1% at 120 min (Table 2).

The experiment conducted with S. khuzestanica showed all of the protoscolices died in 0.1% concentrations. On the other hand, the mortality rate was low by increasing the time of exposure and decreasing concentration. The effects of different concentrations of S. khuzestanica extracts on the viability of E. granulosus protoscolices in different exposure times is in shown Table 3.

DISCUSSION
The surgical treatment of E. granulosus cyst is still the current-
The protoscolicidal activity of different extracts of *Olea europaea* and *Satureja khuzestanica* at 30, 60, and 120 min of exposure times

| Concentrations (%) | Rate of death* (No. dead/No. tested) |
|--------------------|--------------------------------------|
|                    | 30 (min)                             |
|                    | 60 (min)                             |
|                    | 120 (min)                            |

Table 3. Scolicidal effects of different concentrations of *Satureja khuzestanica* after 30, 60, and 120 min of application

| Exposure time (min) | Tests | Concentration% (%) | Mortality rate (%) |
|--------------------|-------|--------------------|--------------------|
|                    |       | 1                  | No. dead/No. examined |
|                    |       | 0.1                | 100.0 (205/205)     |
|                    |       | 0.01               | 100.0 (201/201)     |
|                    |       | 0.001              | 88.9 (185/209)      |
|                    |       | 0.1                | 80.3 (163/203)      |
|                    |       | 0.01               | 82.1 (166/202)      |
|                    |       | 0.001              | 78.2 (159/202)      |
|                    |       | 0.1                | 79.4 (170/214)      |
|                    |       | 0.01               | 79.4 (170/214)      |
|                    |       | 0.001              | 69.1 (146/211)      |
|                    |       | 0.1                | 100.0 (203/203)     |
|                    |       | 0.01               | 100.0 (203/203)     |
|                    |       | 0.001              | 85.5 (177/207)      |
|                    |       | 0.1                | 85.1 (178/209)      |
|                    |       | 0.01               | 79.4 (170/214)      |
|                    |       | 0.001              | 79.2 (172/217)      |
|                    |       | 0.1                | 69.5 (141/203)      |
|                    |       | 0.01               | 74.1 (146/197)      |
|                    |       | 0.001              | 74.1 (146/197)      |
|                    |       | 0.1                | 100.0 (203/203)     |
|                    |       | 0.01               | 100.0 (203/203)     |
|                    |       | 0.001              | 85.5 (177/207)      |
|                    |       | 0.1                | 85.1 (178/209)      |
|                    |       | 0.01               | 79.4 (170/214)      |
|                    |       | 0.001              | 79.2 (172/217)      |
|                    |       | 0.1                | 69.5 (141/203)      |
|                    |       | 0.01               | 74.1 (146/197)      |
|                    |       | 0.001              | 74.1 (146/197)      |

Table 2. The protoscolicidal activity of different extracts of *Olea europaea* and *Satureja khuzestanica* at 30, 60, and 120 min of exposure times

| Concentrations (%) | Rate of deatha (No. dead/No. tested) |
|--------------------|--------------------------------------|
|                    | 30 (min)                             |
|                    | 60 (min)                             |
|                    | 120 (min)                            |

Results are expressed as mean±SD.

*Concentration of all of the plant extract perpetrated with 1 batch.*

*Mortality rate (%)*
tality rate to 67.6% at 120 min exposure time. Low concentrations (0.1%) of *O. europaea* leaf extracts had effective protoscolicidal efficacy. It is remarkable that increasing exposure time showed more scolicidal activities.

In conclusion, the present study is the first report demonstrating the effectiveness of *S. khuzestanica* and *O. europaea* on protoscolices. *S. khuzestanica* had the greatest scolicidal effect against cystic echinococcosis. This plant may be useful as an agent in the PAIR (Puncture-Aspiration-Injection-Respiration) method for cystic echinococcosis because of its rapid and strong scolicidal effects. It seems that *O. europaea* leaf extracts have a less scolicidal activity, but it could be used as an agent with surgery techniques. However, more research is necessary to evaluate mode of actions and in vivo effects of these plant extracts, and also possible side effects on animals and humans.

**ACKNOWLEDGMENTS**

The technical assistance of Ms. Yousra Hosseini and Mr. Arash B. Jamshidi is acknowledged with pleasure. This study was partially funded by a grant (No. HRC-1170) from Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Iran. The authors declare that there is no conflict of interest.

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