

## Full Length Research Paper

# Insignificant activity of *Allium paradoxium* and *Tanacetum parthenium* on protoscoleces of *Echinococcus granulosus*: In vitro study

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Medicinal plants are now recognized as most important alternative for chemical drugs resource, especially for anti-microbial targets. The current study aimed to determine the scolicidal effect of *Allium paradoxium* and *Tanacetum parthenium* as medicinal plants *in vitro*. Protoscoleces were exposed to different concentrations of chloroformic and hydroalcoholic extracts (1, 10, 50 and 100 mg/mL) for 5, 10, 30, 60 and 120 min. Viability of protoscolices was confirmed by 0.1% Eosin staining. The results of this study showed that all extracts used were not significantly effective in comparison with the control group. Therefore, chloroformic and hydroalcoholic extracts of *A. paradoxium* and *T. parthenium* could not be used during hydatid cyst treatment. However, there remains the need to perform advanced comparative clinical studies on the efficacy of *A. paradoxium* and *T. parthenium* and other scolicidal agents. Chloroformic and hydroalcoholic extract of *A. paradoxium* and *T. parthenium*, did not show any scolicidal activity at the concentration of 1, 10, 50 and 100 mg/ml.

**Key words:** *Allium paradoxium*, *Tanacetum parthenium*, *Echinococcus granulosus*, *in vitro*.

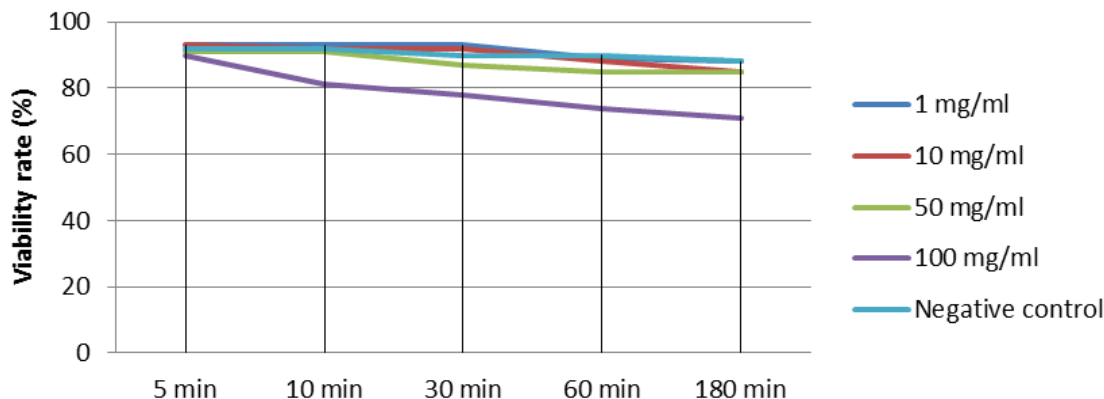
## INTRODUCTION

Hydatid disease due to *Echinococcus granulosus* remains an imperative, challenging medical problem (Brunetti et al., 2010). It is endemic in developing regions because of their poor socioeconomic conditions, but because of travelling and migration, it continues to have a global distribution. Hydatid cysts may be found anywhere in the body, but liver and lungs are the most infected organs (Pawlowski et al., 2001). Surgery is still the chosen method of treatment (Topcu et al., 2006). Repetition during the long-term follow-up period is the most usual problem with this therapeutic technique. For decrease in

the recurrence rate of disease, it is necessary to avoid spilling of the cyst contents and the use of active scolicidal agents (Eckert et al., 2001). The World Health Organization (WHO) regards the use of both effective and safe agents for intraoperative killing of infectious material, but unfortunately; there is no ideal agent up to now. According to WHO reports, therapeutic plants with a relatively low risk are the suggested for traditional treatment of disease (WHO, 1996).

*Allium paradoxium* and *Tanacetum parthenium* are the Iranian native plants that indicate antimicrobial,

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**Figure 1.** The effects of chloroformic extract of *A. paradoxium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

antifungal and antiviral effects. Also, these two plants showed anti-giardial effects *in vitro* (Shirzad et al., 2014). B-Pinene (6.45%), limonene (3.4%), Z-nerolidol (18.01%), spathulenol (22.06%), alpha-bisabolol (5.06%), phytol (9.15%), n-docosane (4.79%) and n-tricosane (3.8%) are the most important identified compounds of *A. paradoxium* (Akbar and Ahmadi, 2010). Camphor and chrysanthenyl acetate are the main components of flower heads of *T. parthenium* (Mostafa et al., 2007). Considering the low range of scolicidal compounds, searching for a new scolicidal agents, particularly from natural sources is of great interest (Rahimi-Esboei et al., 2012; Gholami et al., 2013).

Since *A. paradoxium* and *T. parthenium* has been shown to have a number of medicinal and anti-parasitic properties and considering the origin of these plants to Iran, the present study aimed to assess the scolicidal effect of the chloroformic and hydroalcoholic extracts at different concentrations and for various exposure times.

## MATERIALS AND METHODS

### Parasite

Hydatid cysts of *E. granulosus* were detached from livers of naturally infected sheep slaughtered at Mazandaran abattoir, northern part of Iran under aseptic conditions. The fluid of hydatid cyst containing protoscoleces was removed by a 50 ml syringe and transferred into a 50 ml Falcon tube. After 30 min of immovability, settled protoscoleces were washed three times with normal saline (pH 7.2). The viability of the parasites was assessed with 0.1% eosin under a light microscope (400x) (Gholami et al., 2013).

### Preparation of extracts

The sliced seeds of *A. paradoxium* and the aerial parts of *T. parthenium* were dried under shade and powdered mechanically using a commercial electric blender. Dried powder (100 g) were extracted by percolation method with hydroalcohol and chloroform

solution, separately for 72 h in room temperature. The obtained solution was passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. Finally, the extracts were concentrated using a rotary evaporator (Heidolph, Germany), placed in a sterile glass and stored at -4°C for later use (Shirzad et al., 2014).

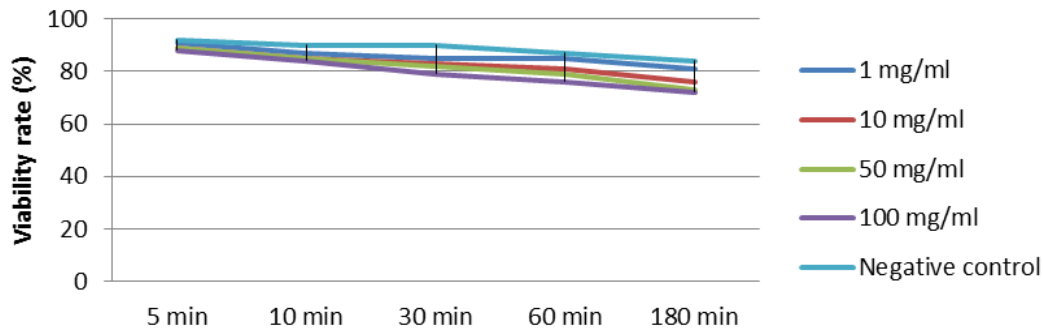
### Scolicidal tests

The scolicidal tests were done three times (triplicate) and based on Rahimi-Esboei et al. (2013). In the current study, four concentrations (1, 10, 50 and 100 mg/ml) of extracts were examined for 5, 10, 30, 60 and 180 min. 2 ml of each concentration was transferred to a test tube; 50 µl of sediment protoscoleces was added to the tube and mixed lightly. The tube was then incubated at 37°C for 5, 10, 30, 60 and 180 min. At the end of each incubation time, viability of the protoscoleces was evaluated by 0.1% eosin stain (Rahimi-Esboei et al., 2013).

## RESULTS

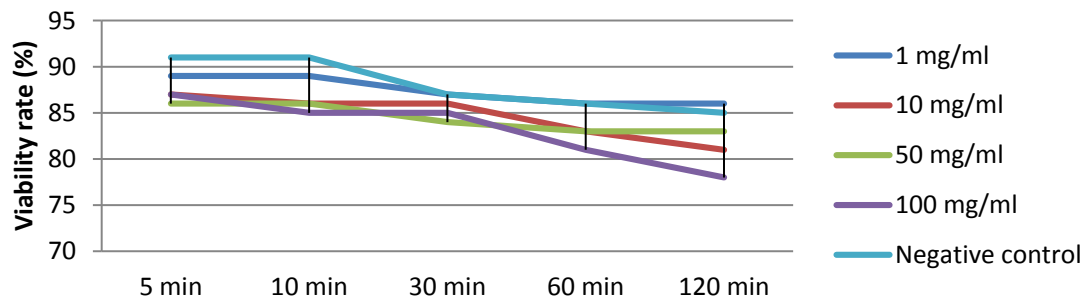
Results of the effectiveness of different concentrations of *A. paradoxium* and *T. parthenium* extract as a scolicidal agent are shown in Figures 1 to 4. While the viability rate in the control group was 88% after 180 min, scolicidal activity of chloroformic extract of *A. paradoxium* extract at concentration of 100 mg/mL was 90, 81, 78, 74 and 71% after 5, 10, 30, 60 and 180 min of application, respectively (Figure 1). Scolicidal effect of hydroalcoholic extract of *A. paradoxium* extract at concentration of 100 mg/mL was 88, 84, 79, 76 and 72% after 5, 10, 30, 60 and 120 min of exposure, respectively (Figure 2). While the viability rate in the control group was 85% after 180 min, scolicidal activity of chloroformic extract of *T. parthenium* extract at concentration of 100 mg/mL was 87, 85, 85, 81 and 78% after 5, 10, 30, 60 and 180 min of application, respectively (Figure 1).

Scolicidal effect of hydroalcoholic extract of *T. parthenium* extract at concentration of 100 mg/mL was



**Figure 2.** The effects of hydroalcoholic extract of *A. paradoxium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

### Chart Title



**Figure 3.** The effects of chloroformic extract of *T. parthenium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

84, 78, 78, 73 and 69% after 10, 20, 30 and 40 min of exposure, respectively (Figure 2).

In this study, the effectiveness of two plants were increased by increasing the time and concentration of plants, but there was no significant differences between times of exposure and concentration of extracts.

## DISCUSSION

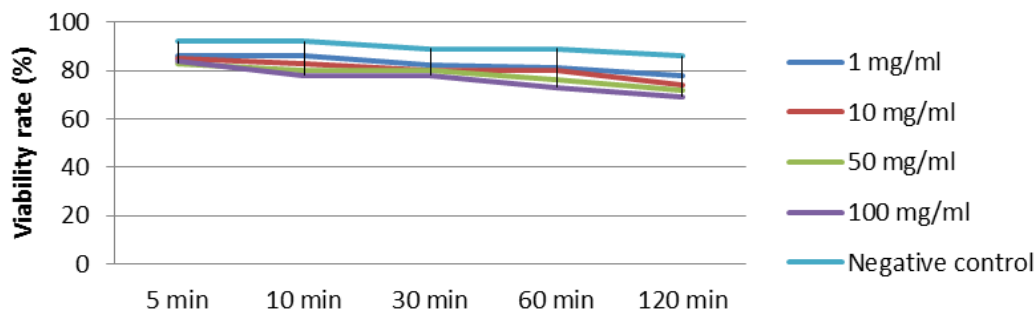
The current work examined the scolical activity of *A. paradoxium* and *T. parthenium* using the chloroformic and hydroalcoholic extracts and an *in vitro* technique. Shirzad et al. (2014) showed high effectiveness of *A. paradoxium* and *T. parthenium* on *Giardia lamblia* in *in vitro* investigation with concentration of 100 mg/ml after 3 h. Also, this anti-giardial activity of *A. paradoxium* was reported by Elmi et al. (2014) in an *in vivo* study. Despite the anti-bacterial and anti-parasitic activity of these plants, the results of this study indicate insignificant efficacy against protoscoleces of hydatid cyst (Shirzad et

al., 2014). It could be concluded that protoscoleces of hydatid cyst are more resistance than other used microorganisms and it depends on the physiological structure of cells. In this study, the used plants were inactive against protoscoleces of hydatid cyst. Perhaps, a longer exposition time was needed while the same concentration and exposure time was effective against some microbial and parasitic infections (Sarkari et al., 2009; Mehrabi et al., 2011; Radwan et al., 2012).

This *in vitro* technique is simple but confirmed, trustworthy, sensitive and extensively used (Dey, 1980). Therefore, the results gained are permissible and reliable. As shown (Figures 1 to 4), the chloroformic and hydroalcoholic extracts of *A. paradoxium* and *T. parthenium* did not exhibit any *in vitro* scolical activity, even at concentrations of 100 mg/ml after 180 min. From the extracts and plants, hydroalcoholic extract of *T. parthenium* was the most effective extract in concentration of 100 mg/ml after 180 min.

This study strongly recommends evaluating the activity with a longer period and develops more research on the

Chart Title



**Figure 4.** The effects of hydroalcoholic extract of *T. parthenium* on viability of protoscolexes of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

phytochemistry of these plants.

### Conflict of Interests

The authors have not declared any conflict of interests.

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