

# The Anthelmintic Effect Of *Urtica dioica* And *Tanacetum vulgare* L. On Protoscoleces Of *Echinococcus granulosus*

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## Abstract

The current study evaluated the effects of alcoholic (Ethanol) extracts and aquaus extract of leaves *Urtica dioica* and extract of both leaves and flowers of *Tanacetum vulgare* on the viability of *Echinococcus granulosus* protoscolices in vitro. Three different concentrations of each extract (1, 2, 4 micrograms /ml) were used. The mortality of Protoscoleces of *Echinococcus granulosus* by using aqueous extract of two plants was increased with the increasing the concentration and duration of exposure; reach 96.2% and 97.8% at concentration of 4 micrograms/ml for 30 minutes for *Urtica dioica* and *Tanacetum vulgare* respectively. However, it was found that the effect of these plants by ethanol were decreased with the same concentration when the time of exposure is increased. The mortality rate of protoscoleces decreased from 69% to 4.2% when exposed to *Urtica dioica* extract for 10 and 30 min respectively at 1mg/ml.

**Keywords:** *Echinococcus granulosus*, cystic echinococcus, protoscolex, *Urtica dioica*, *Tanacetum vulgare* Kurdistan Region.

## 1. Introduction

Several medicinal plants have been traditionally used as remedies against different disease. The uses of herbal medicines have been increased recently [1].

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Majority of the screening reports were in vitro studies which used some worm samples such as Indian earthworm (*Pheretima posthuma*), *ascaridia galli*, *Ascaris lumbricoids* and protoscoleces of *Echinococcus granulosus* because of their availability [2]. The alkaloids, sugars, saponins, aromatic oils, resins and other are the most important substances that could act as anthelmintic effect [3]. *Echinococcus granulosus*, *E. Oligarrthua*, *E. multiocularis*, *E.vogeli* and *E. shiquicus* are species of *Echinococcus* genus [4,5]. The most widespread species is *E. granulosus* from family Taeniidae which cause echinococcosis (cystic hydatid disease), affecting both humans and animals [6,7]. Tap worm eggs are passed through the feces of infected carnivores and may subsequently infect human who accidentally ingest them [8]. Tumor-like growths that occur mostly in liver and lung, with varying degrees of infestation of other organs are clinical signs of hydatidosis [9].

The extract of two plant *Olea europaea* and *Satureja khuzestanica* were used as scolicidal effect studied by author [10] Found that 0.1 and 0.01 µg/ml concentration had strong scolicidal effects in 30, 60 and 120 min and the rate of mortality decreased with the lower concentration.

[11] studied the effects of different concentration of alcoholic extract of *salvia officinalis*, *Thymus vulgaris* on the viability of *echinococcus granulosus* in vitro and they found that the concentration of 2, µg/ml of both extracts have a significant protoscolicidal activity within the 6<sup>th</sup> day. In addition the warm water at 50-60 °C can be regarded as a scolicidal agent [12].

[13] used protoscolicidal effect of garlic (*Allium sativum*) extract on protoscoleces of hydatid cysts in vitro and they found the protoscolicidal activity increased. [14] when he used scolicidal effects of *corylus spp*, Seeds and hazel of *Curcubia spp.*, nut and garlic extracts, he indicated that the garlic had more potential scolicidal effects among all the 3 plant and he was also found that the chloroformic extract of garlic was the most potential protoscolicidal among all of the extracts and were killed 98% of protoscolices in 50mg/ml within minimum of 20 minutes of exposure.

*Urtica dioica L.* (nettle) belongs to the plant family Urticaceae, with a height of 50-150 cm. and found in Barwry balla area located at the north east of Duhok Governorate-Kurdistan Region. The *Urtica dioica* (Nettle) is herbaceous perennial flowering plants, which is a member of the Urticaceae family [15].

This plant contains chlorophyll, vitamin C, vitamin K, Panthotene acid, carotenoids, B group vitamin (B1, B2), tannins, essential oil, proteins, and minerals (Fe,Cu,Mn,and Ni). Acetylcholin and histamine [16,17]

And **Tanacetum vulgare L.** commonly known as Tansy, is a herbaceous plant growing in temperate Europe and Asia this is found in Barwry Balla area Duhok Governorate –Kurdistan Region [18]. Various preparations of tansy are known to be used for treating rheumatic pain, skin eruption, and diuretic conditions [18, 19, 20, 21].

Tansy has also been reported as an anthelmintic, antihypertive, and antiseptic, antihypertensive and as an antispasmodic agent [22]

The aim of this study was to investigate the in-vitro effect of the Ethanol and Aqueous Extract of *Urtica dioica* and *Tanacetum vulgare* on Protoscolices of *Echinococcus granulosus*.

## **2. Materials And Methods**

### *2.1 Plant material*

Leaves of *Urtica dioica* and leaves and flowers of *Tanacetum vulgare* were collected from Barwary Balla area exactly from Binavi village which is located at the north east of Amedi province at the Turkey border in north of Duhok city –Kurdistan Region , this plant identified and classified by Dr.Prof Salim shahbaz , Faculty of Agriculture and forestry University of Duhok . The plants were dried in open air and shady conditions until completely dried and then ground to a powder.

#### *Ethanol extraction:-*

Dried leaves of *Urtica dioica* and leaves and flowers of **Tanacetum vulgre** were powdered mechanically using a commercial electrical blender. 25 g of dry powder was added to 250 ml of pure ethanol were extracted by using a soxhlet extraction apparatus. The residue was dried overnight and extracted with water at 60°C. The ethanol extracts were evaporated using a rotary evaporator and the water extracted with a freeze-dryer. The dried crude extracts were kept in dark plastic bags, sealed under vacuum, and kept in a freezer at -20°C until the time of use according to [23]

#### *Preparation of aqueous plant extracts*

Water extract was prepared by boiling 100 gm of the plant powder in 200ml of distilled water, mixed up well and shake for 15 min. the mixture was filtered through a Whatman No.1 filter paper according to [24]

### *2.4 Collection of protoscolices*

Hydatid cysts of *Echinococcus granulosus*, were collected from the liver and lungs of infected sheep slaughtered at Duhok slaughtered house and carried to the Research Laboratory at the Faculty of Veterinary Medicine, University of Duhok . The protoscolices were obtained from the hydatid fluid and washed 3 times in normal saline and Ring solution. And the protoscolices keep in a saline solution with hydrated fluid according to [11]

### *2.5 Viability assay*

The viability was tested microscopically after adding 10 µl of eosin solution to 10 µl of protoscolices for 15 min. (0.1 mg of eosin stain was dissolved in 100 ml distilled water). Stained protoscolices were considered as dead while unstained protoscolices were recorded as a live.

## 2.6 Statistical analysis

The statistical analysis was performed with the use of SPSS.

## 3. Results and Discussion

Table (1):- show the effect of aqueous and ethanol extract of *Urtica dioica* on protoscolices

Concentration	Duration	Aqueous extract of <i>Urtica dioica</i>	Ethanol of <i>Urtica dioica</i>
1 microgram	10 minutes	13.8% killed	69%
2 microgram	10 minutes	37.5%	63.4%
4 microgram	10 minutes	46%	49.9%
1 microgram	20 minutes	46%	28.3%
2 microgram	20 minutes	49.5%	58.3%
4 microgram	20 minutes	63%	58.5%
1 microgram	30 minutes	75%	4.2%
2 microgram	30 minutes	94.9% *	32%
4 microgram	30 minutes	96.2% *	34.3%

- \*  $P \geq 0.05$

Table ( 2 ):- show the concentration, duration and method of Extraction of *Tanacetum Vulgare*

Concentration	Duration	<i>Tanacetum vulgare</i> water	<i>Tanacetum vulgare</i> Ethanol
1 microgram	10 minutes	7.5% Killed *	24.3%
2 microgram	10 minutes	25.89%	18.9%
4 microgram	10 minutes	57.8%	17.6%
1 microgram	20 minutes	65.5%	28.3%
2 microgram	20 minutes	76.3%	58.3%
4 microgram	20 minutes	79%	58.5%
1 microgram	30 minutes	95.3% *	37.5%
2 microgram	30 minutes	97.5%	18.5%
4 microgram	30 minutes	97.8%	10.5

- \*  $P \geq 0.05$

The use of plant extracts against Hydatid cyst has received a critical attention recently. Some studies indicated that extracts of certain plant species, belonging to different families may affect the viability of protoscolices and the survival of secondary hydatid cysts [25].

The effect of aqueous and ethanol extract of *Urtica dioica* and *Tanacetum vulgare* on protoscolices of *E. granulosus* is shown in Table (1 and 2).

In study it was found after 30 minutes of exposure the protoscolices of *E. granulosus* to 4µg/ml of aqueous extracts of *Urtica dioica* and *Tanacetum vulgare* induced a significant decrease in the viability of protoscolices.

By *Urtica dioica* was 96.2% and by *Tanacetum vulgare* was 97.8%. However, the exposure protoscoleces to ethanol extract of *Urtica dioica* was 69% at concentration 1 µg/mL for 10 minutes. And the mortality was 58.5% after exposure to ethanol extract *Tanacetum vulgare* for 20 minutes at concentration 4 µg/mL.

The less effective of aqueous extract of *Urtica dioica* was 13.8% at concentration 1 µg /mL for 10 minutes and by ethanol extract 1 µg /ml for 30 minutes was 4.2%.

*Tanacetum vulgare* has less effect were by aqueous extract 1micrograms for 10 minutes (7.5%) and 4micrograms/ml for 30 minutes (10.5%) by ethanol extract.

The result of ethanol extract of *Urtica dioica* leaves agreement with what found by [26] when they used *Urtica piluifera* at same time the aqueous extract of this plant disagree with the same authors.

Also the effect of aqueous of both plant agreement with what have been discussed by [27] according to the parasites model used, life stage of the parasite, and host animal tested, it is clear that polar extracts obtained with water or ethanol have a greater anthelmintic activity than non-polar extracts obtained with hexane which usually have low or no anthelmintic activity.

*Urtica dioica* and *Tanacetum vulgare* are widely used as remedies against different disease in Kurdistan [26]. But we could not find any knowledge about anthelmintic activity of *Urtica dioica* to discuss. Anthelmintic activity of the leaves of *Urtica dioica* and leaves and flowers of *Tanacetum vulgare* were investigated in this study. Revealed that the aqueous extract of *Urtica dioica* and *Tanacetum vulgare* have a significant anthelmintic activity. While, the ethanol extract of both plant has less effect.

#### 4. Conclusion

Because in most reference these two plants classified under poisoning plant the uses it should be very careful. And the best concentration for short it should be 1 µg/ml. for 10 minutes from ethanol extract of *Urtica dioica*.

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