

***In vitro* Scolicidal Effect of Ginger (*Zingiber officinale* Roscoe) Ethanolic Extract Against Protoscolices of Hydatid Cyst**

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Abstract:

BACKGROUND: To prevent the recurrence of hydatid cysts after surgery, it is essential to use effective scolicidal agents. Most of these agents are not safe due to their undesired side effects. Recently, studies have been conducted to find natural scolicidal agents with more efficacies and low side effects.

OBJECTIVES: In this study, the *in vitro* scolicidal effect of ethanolic extract of ginger (*Z. officinale*) on protoscolices of hydatid cyst was investigated.

METHODS: A certain number of protoscolices (about 500) were treated with various concentrations of ginger ethanolic extract (40, 50, 100, 150 and 200 mg/ml) and the effect of each concentration was evaluated for specified time periods (15, 30, 45 and 60 minutes). Each concentration/time was performed in triplicate and the viability of protoscolices was confirmed by 0.1% eosin staining.

RESULTS: The mortality rate with ethanolic extract of *Z. officinale* after 60 minutes was as follows: 68%, 92.3%, 93% and 100% at 50, 100, 150 and 200 mg/ml, respectively, and there was no significant difference between the three concentrations of 200, 150 and 100 mg/ml ($P>0.05$). However, at the concentrations of 200 mg/ml after 30 minutes of incubation, 100% protoscolices were dead. Based on Tukey's test, a significant difference in the percentage of live protoscolices was found between the different concentrations of ginger extract with the exception of between 150 and 100 mg/ml concentrations ($P>0.05$).

CONCLUSIONS: Ethanolic extract of *Zingiber officinale* had a high scolicidal activity *in vitro*, and it has the potential to be used as a scolicidal agent in the surgical treatment of hydatid cysts.

Keywords:

Ethanolic extract, Ginger, Hydatid cyst, *In vitro*, Scolicidal effect

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Introduction

Treatment of hydatid cyst is often expensive and complicated and sometimes it requires extensive surgery and/or prolonged drug therapy. If surgery is carried out without complete and effective anti-infective treatment, frequent relapses will occur. Approximately 6.5% of cases experience relapse after an intervention; therefore, prolonged recovery time is required. Annual costs associated with cystic echinococcosis (CE) are estimated to be US\$ 3 billion for treating cases and losses to the livestock industry can include liver condemnation, reduction in carcass weight, decrease of milk production and reduced fertility (WHO, 2018).

This multi-host disease is one of the most important public health infectious diseases in Iran (Sadjjadi, 2006; Rokni, 2008). Sheep that present with a 35.21% infection rate and 88% fertilized cysts have been considered as the most important intermediate host in Iran (Fasihi Harandi, 2002; Rokni, 2009). Fasihi Harandi (2012) suggested that the overall annual cost of CE in Iran was estimated to be US\$232.25 million, of which US\$93.39 million and US\$132.0 million were attributed to human CE and the total economic losses due to livestock CE in Iran, respectively.

Surgery is one of the best choices for the treatment of hydatidosis and to prevent relapse, effective scolicidal agents must be used after surgery (Brunetti, 2010). There are several agents, which have been used for inactivation of the cyst contents, for example, hypertonic saline, silver-nitrate, cetrimide, and ethanol, but various side effects such as sclerosane colangitis (biliary tract fibrosis), liver necrosis and methaemo-

globinaemia have been reported following their use (Mahmoudvand, 2014a). At present, research is attempting to find a natural scolicidal agent with increased efficacy and low side effects. Herbs are very important and useful therapeutic agents against many pathological infections. Ginger (*Zingiber officinale* Roscoe) of the family Zingiberaceae, is one of these herbs whose rhizome has also been used in traditional herbal medicine (Park, 2002; Sivasothy, 2011). The characteristic fragrance and flavor of ginger result from volatile oils that compose 1-3% of the weight of fresh ginger, primarily consisting of zingerone, shagaols, and gingerols as the major pungent compound (An, 2016). Many studies have shown that ginger has antibacterial (Gao, 2010; Malu, 2009; Sebiomo, 2011), antifungal (Ahmed, 2012; Aghazadeh, 2016) and antiparasitic effects (Arbabi, 2016; Mekuriya, 2018; Wonhyung, 2013). The aim of this study was to evaluate the effect of ginger ethanolic extract against protoscolices of hydatid cyst, to explore the potential of ginger as natural scolicidal agent.

Materials and Methods

Ginger extraction: First, ginger rhizomes (*Zingiber officinale*) bought from the traditional Bazar of Rasht in the north of Iran, were washed with sterile water and then placed in the open air for 72 h to dry completely. The rhizomes were then cut into thin slabs and placed on clean gauze to dry completely. After drying the ginger, these sheets were milled to obtain a homogeneous ginger powder. For extraction (Baquer, 2014), 100 g of powder was dissolved in 500 ml of 70 % ethanol as much as possible under the action of a magnetic

Table 1. Comparison of protoscolices viability in ethanolic extract of Ginger according to time and concentration by Tukey's multiple comparison test. Mean=M, Standard deviation=SD, Positive Control= C+, Negative Control= C-. (a, b, c, and d indicate no significant difference ($P \geq 0.05$) in Tukey's test).

Concentration (mg/ml)	Time (minutes)				
	15 M± SD	30 M± SD	45 M± SD	60 M± SD	Total M± SD
C-	79.67 ^a ± 5.912	76.07 ^a ± 6.147	71.06 ^a ± 6.685	67.46 ^a ± 4.583	73.565 ^a ± 8.087
40	73.33 ^a ± 2.082	67.00 ^a ± 2.646	60.66 ^a ± 2.517	56.66 ^a ± 4.163	64.42 ^a ± 8.306
50	55.00 ^{a,b} ± 9.644	43.33 ^b ± 3.512	38.33 ^b ± 4.726	32.00 ^b ± 6.928	42.17 ^b ± 6.028
100	38.00 ^{b,c} ± 16.823	25.00 ^c ± 3.606	18.66 ^c ± 2.082	7.66 ^c ± 2.517	22.33 ^c ± 9.517
150	27.33 ^{c,d} ± 2.887	20.66 ^c ± 4.041	11.66 ^c ± 6.110	7.00 ^c ± 5.568	16.66 ^c ± 4.805
200	4.00 ^d ± 3.606	0.00 ^d ± 0.000	0.00 ^d ± 0.000	0.00 ^d ± 0.000	1.00 ^d ± 6.562
C+	0.00 ^d ± 0.000	0.00 ^d ± 0.000	0.00 ^d ± 0.000	0.00 ^d ± 0.000	0.00 ^d ± 0.000

stirrer for two hours. The solution obtained was placed in an Erlenmeyer at room temperature for 48 h. After 48 h, the solution was filtered (Whatman paper No.1). The filtered solution was then placed in an oven for evaporation at 37 °C, and finally the dried extract was kept at 4°C until it was used in a sterile container.

Preparation of protoscolices: At this stage, ovine and caprine liver and lung samples contaminated with hydatid cysts were collected from Rasht slaughterhouse and immediately transferred to the laboratory. The fluid in the cysts containing protoscolices was drained using sterile syringes and placed in tubes in an incubator at 37 °C. After the protoscolices were deposited, the supernatant fluid was removed, and finally, a thick liquid containing protoscolices was obtained.

Scolicidal test: Approximately 20 µl of sediment containing about 500 protoscolices were treated with various concentrations of ginger extract (40, 50, 100, 150 and 200 mg/ml) and the effect of each concentration was evaluated across different specified time periods (15, 30, 45 and 60 min). Each tube was incubated at 37 °C and removed at the allocated time (15, 30, 45 and 60 min) for performing the subsequent tests. Each

concentration/time was undertaken in triplicate and saturated saline and normal saline were used as positive and negative control, respectively in each stage (Mahmoudvand, 2014b).

Viability test: At the end of each concentration/time, after removing the supernatant with sampler, one ml of 0.1% eosin solution was added to each microtube and mixed before being incubated for 15 min. The precipitates of each microtube were then expanded and protoscolices were examined under light microscopy (100x magnification) and the number of live and dead protoscolices were counted.

Statistical analysis: For each concentration/time, the numbers of live and dead protoscolices were counted to enable percentages for each sample and the mean percentage across the three samples to be calculated. SPSS software was used to analyze data and Tukey's test was performed to compare the means of the different concentrations for each time period examined. Significance was set at $P < 0.05$.

Results

In the viability test, the live protoscolices are not stained with eosin, while the dead protoscolices are stained (Fig. 1). Based on

Table 2. Summary of previous research examining the effect of herbs on protoscolices within hydatid cysts. In High Efficacy column, M= Mortality rate and everywhere that M has not been written, it is 100%.

Botanical name	General name	Researcher Year	Extract type	High Efficacy
<i>Allium sativum</i>	Garlic	Sadjadi (2009)	chloroformic (cloves)	100 mg/ml: 4 h 200 mg/ml: 30min
		Moazeni (2010)	methanolic (cloves)	25 mg/ml: 60 min 50 mg/ml: 10 min
		Eskandarian (2012)	chloroformic (cloves)	50 mg/ml: 20 min M: 98%
		Rahimi-Esboei (2016)	ultrasonic methanolic (flowers)	100 mg/ml: 180 min
		Salehi (2014)	aqueous (fruit) hydroalcohol (fruit)	4 mg/ml: 5 min 2 mg/ml: 5 min
<i>Berberis vulgaris</i>	Barberry (Zereshk)	Rouhan (2013)	aqueous (fruit)	4mg/ml: 5 min 2 mg/ml: 30 min M: 98.7%
		Mahmoudvand (2014b)	methanolic (root)	1 mg/ml: 30 min 2 mg/ml: 10 min
<i>Bunium persicum</i>	Black Zira (earthnut) (seeds)	Mahmoudvand (2016a)	essential oil	6.25 µg/ml: 30 min 12.5 µg/ml: 10 min 25 µg/ml: 5 min
<i>Cuminum cyminum</i> L.	Cumin (green Zira) (seeds)	Keyhani (2017)	essential oil	25 µg/ml: 20 min 50 µg/ml: 10 min
			methanolic	50 mg/ml: 20 min 100 mg/ml: 10 min
<i>Dendrosicyos socotrana</i>	Cucumber tree (leaves)	Barzinji (2009)	methanolic	5000 µg/ml: 360 h
<i>Ephedra major</i>	joint-pine	Zibaei (2017)	methanolic (different parts)	0.1% : 60 min
			aqueous (leaves)	5000 µg/ml: 408 h
<i>Eucalyptus globulus</i>	Blue gum (leaves)	Feizi (2015)	methanolic	50 mg/ml: 40 min
		Lashkarizadeh (2015)	Essential oil hypertonic saline	1 msg/ml: 5 min 20% : 10 min
<i>Foeniculum vulgare</i> Mill	Fennel (seeds)	Mahmoudvand (2016c)		50 mg/ml: 30 min 100 mg/ml: 20 min 200 mg/ml: 10 min
				200 µg/ml: 48h
				M<50% 200 µg/ml: 48h M: 50%
<i>Jatropha unicostata</i>	Sibru (leaves)	Barzinji (2009)	methanolic	1000 µg/ml: 288 h
			aqueous	1000 µg/ml: 360 h
<i>Myrtus communis</i> L.	Common myrtle (leaves)	Mahmoudvand (2016b)	essential oil	50 µl/ml: 10 min 100 µl/ml: 5 min

the results, as the concentration of the ginger extract increased, the viability of pro-

toscolices decreased. The highest percentage of viability was observed at 40 mg/ml

Table 2. Contd...

Botanical name	General name	Researcher Year	Extract type	High Efficacy		
<i>Nigella sativa</i>	Black cumin (seeds)	Mahmoud vand (2014c)	essential oil	10 mg/ ml: 10 min		
			methanolic	50 mg/ml: 10 min		
			aqueous	50 mg/ml: 30 min		
<i>Olea europaea</i>	Olive (Leaves)	Zibaei(2012)	aqueous	0.1% : 120 min		
			Zibaei (2016)	hydroalcoholic (fruit & Leaves)	0.1% : 360 min	
<i>Pistacia atlantica</i>	Persian turpentine	Mahmoudvand (2016d)	methanolic (fruit)	25 mg/ ml: 20 min 50 mg/ ml: 10 min		
			Mahmoudvand (2016e)	essential oil methanolic	50 mg/ml: 20 min 100 mg/ml: 10 min	
<i>Pistacia vera</i>	Pistachio (fruit)	Mahmoudvand (2016f)			essential oil	100 mg/ml: 10 min 200 mg/ml: 5 min
<i>Rhus coriaria</i>	Sumac (fruit)	Moazeni (2012a)	methanolic	10 mg/ml: 30 min 30 mg/ml: 20 min 50 mg/ml: 10 min		
				Abdel-Baki (2016)	ethanolic	30 mg/ ml: 30 min 50 mg/ ml: 20 min
						Gholami (2013)
<i>Satureja khuzestanica</i>	Satureja (Leaves)	Zibaei (2012)	hydroalcoholic	0.1% : 30 min		
<i>Trachyspermum ammi</i> L.	Ajowan (fruit)	Moazeni (2012b)	essential oil	5 mg/ml: 60 min 10 mg/ml: 10 min		
				Kavoosi (2013)	essential oil	0.017 mg/ml: 10 min
		Moazeni (2012c)	methanolic			10 mg/ ml: 3 min 25 mg/ ml: 1 min
				<i>Zataria multiflora</i> Bioss	Shirazi thyme (Leaves)	Jahanbakhsh (2015)
Moazeni (2017)	nano emulsion of essential oil	1 mg/ ml: 20 min 2 mg/ ml: 10 min				
		Moazeni (2011)	methanolic			
<i>Zingiber officinale</i>	Ginger (rhizome)			Baqer (2014)	ethanolic	50 mg/ml: 120 min 100 mg/ml: 90 min 150mg/ ml: 60 min
		Feizi (2015)	methanolic			100 mg/ml: 40 min
						Shahnazi (2016)

of *Z.officinale* extract and the lowest survival level was found at 200 mg/ml. As shown in Fig. 2, 100% mortality rate with ethanolic extract was observed at the concentrations of 200 mg/ml after 30 min of incubation. The mortality rate of the ethanolic extract of *Z.officinale* at concentration of 150 mg/

ml was 72.7, 79.4, 88.4 and 93% after 15, 30, 45 and 60 min of incubation, respectively. These values for the concentration of 100 mg/ml were 62, 75, 81.4 and 92.4%, respectively. On the other hand, at the concentration of 50 and 40 mg/ml, after 60 min, 32 and 56.6% protoscolices were still alive.

Mean and standard deviation of protoscolicidal effects of ginger ethanolic extract and positive and negative controls in different times and concentrations are provided in Table 1. A significant difference in the percentage of live protoscolices was found between the different concentrations of ginger extract ($P < 0.05$), with the exception of between two concentrations: 150 and 100 mg/ml ($P > 0.05$). A significant difference in the percentage of live protoscolices also occurred between the different treatment times with ginger extract ($P < 0.05$). At 200 mg/ml concentration of ginger extract, there was no significant difference in the percentage of protoscolices at different times ($P < 0.05$). Moreover, the scolicidal power of saturated saline as the positive control was 100% after 15 min of application. Therefore, the scolicidal activity of ethanolic extract at the concentration of 200 mg/ml after 30 min was similar to the scolicidal activity of positive control. The scolicidal effect of various concentrations of the ginger ethanolic extract, except 40 mg/ml concentration was extremely significant ($P < 0.05$) compared to the control group (normal saline) at all exposure times. In the 60th min, no significant difference was found between the three concentrations of 200, 150 and 100 mg/ml ($P > 0.05$).

Discussion

Because of the low success rate of drug therapy, surgery is the best approach for treatment of hydatid cyst, but this method also has its own problems and to prevent surgery complications, the use of medication after surgery is common. Given the pathophysiological importance of hydatid cyst, researchers have always tried to find a suitable and effective way to control and

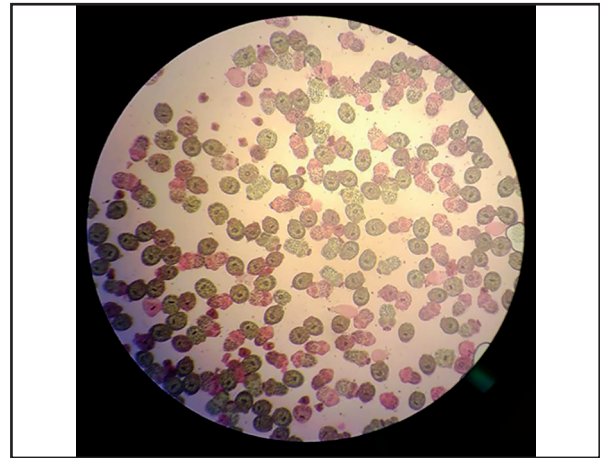


Figure 1. The live and dead protoscolices under the light microscope, the live protoscolices have not been stained, while the killed protoscolices are stained with Eosin.

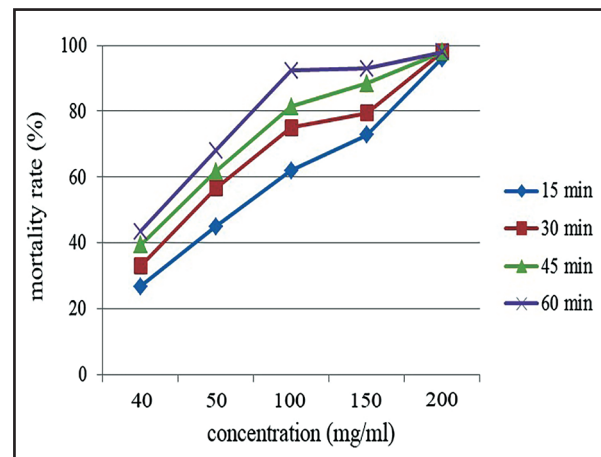


Figure 2. In vitro lethal effects of methanolic extract of *Zingiber officinale* against protoscolices of hydatid cyst at the various concentrations following various exposure times.

treat the hydatid cyst. The use of medicinal herbs is one potential treatment option. Herbal extracts have fewer side effects than conventional treatments and might be more effective. Many medicinal herbs have been used to prevent and treat parasites in the world and the study of the effect of different herbs on protoscolices have also yielded satisfactory results. In this study, we investigated the effect of ginger root extract on the viability of hydatid cyst protoscolices.

In the current study research, at 40 mg/ml concentration of ginger extract, even af-

ter 60 min, 56.7% of the protoscolices were alive. As the concentration of the extract increased, the percentage of viable protoscolices decreased. At 50, 100 and 150 mg/ml concentrations of ginger extract, after 60 min, 32%, 7.7% and 7% protoscolices were alive, respectively. In contrast, ginger extract at the concentration of 200 mg/ml killed 100% of protoscolices in 30 min and only very few (4% average) survived at the 15 min. Accordingly, the concentration of 200 mg/ml had the highest effect, followed by 150, 100, 50 and 40 mg/ml, respectively and based on Tukey's test, no significant difference was found between the two concentrations of 100 and 150 mg/ml. The greatest effect was observed in the 60 min and the lowest in 15 min.

The research on affective medicinal herbs on hydatid cyst has increased in recent decades, and most of these studies have been conducted in Iran (refer to Table 2). Numerous plants, such as extract of onion, *Allium cepa* and basil, *Ocimum bacilicum* (Haghani, 2014), *Artemisia aucheri* (Feizi, 2015), aqueous and hydro-alcoholic extracts of garlic (Sadjadi, 2009), squash seeds and hazel nut (Eskandarian, 2012) are reported as ineffective in the treatment and prevention of hydatid cysts. Herbs have also been investigated and can be effective on protoscolices, but after a several days, such as *Dendrosicyos socotrana* and *Jatropha unicostata* (Barzinji, 2009), *Mentha piperita* (Maggiore, 2012), *Salvia officinalis*, *Origanum vulgare* (Pensel, 2014) and *Thymus vulgaris* (Doaa, 2011; Pensel, 2014).

The most effective herb on protoscolices is Black Zira (*Bunium persicum*), only 25 µg/ml essential oil from its seeds can destroy one hundred percent of protoscolices in five min (Mahmoudvand, 2016a). The

seeds of Green Zira and the leaves of Common Myrtle are also reported to be effective with a 100% mortality rate on protoscolices in dosage of 50 µg/ml essential oil at 10 min (Keyhani, 2017; Mahmoudvand, 2016b).

Heretofore, three studies have examined ginger, *Zingiber officinale*. Moazeni (2011) and Feizi (2015) found 100mg/ml methanolic extract of ginger destroyed one hundred percent of protoscolices in 30 and 40 min, respectively.

The only study on ethanolic extract of ginger was performed by Baqer (2014) who reported that in concentrations of 50 and 100 mg/ml ginger extract, 46% and 37.5% of protoscolices were alive after 30 min and 15.3% and 20% protoscolices were alive after 45 minutes. However, ginger extract in 50, 100 and 150 mg/ml concentrations, destroyed 100% of protoscolices in 120, 90, and 60 minutes, respectively.

Although in this study the time was not checked after 60 min, 7% of the protoscolices were still alive in concentration of 150 mg/ml at 60 min and these conclusions are inconsistent with the result of the previous investigation (Baqer, 2014). This disparity may be due to the difference in the strains of the parasite in the two regions.

Based on Moazeni (2011) and Feizi (2015), 100 mg/ml methanolic extract of ginger destroys 100% of protoscolices at 30 and 40 min, respectively. Comparing these results with our results suggests that methanolic extract of ginger is stronger than its ethanolic extract.

Main mechanisms of scolicidal effects of ginger are not clear and further studies are needed to elucidate these mechanisms. Although Bahmani (2013) showed that ginger causes the paralysis and death of leeches and Lin (2014) suggested that Ginger

has the cestocidal activity or ability to halt spontaneous parasite movement.

Ginger has few side effects (Marcello, 2001) and it has been introduced by the Food and Drug Administration (FDA) as a healthy substance. Based on the results of the present study, ethanolic extract of *Zingiber officinale* had a high scolicidal activity in vitro. However, further investigation on the in vivo efficacy of *Zingiber officinale* extract and its possible side effects is needed.

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Conflicts of interest

The author declared no conflict of interest.

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اثر اسکولکس کشی عصاره اتانولی زنجبیل (*Zingiber officinale Roscoe*) بر روی پروتواسکولکس های کیست هیداتید

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چکیده

زمینه مطالعه: برای جلوگیری از عود کیست هیداتید پس از جراحی، استفاده از مواد اسکولکس کش موثر ضروری است. اکثر این مواد به دلیل عوارض جانبی ناخواسته شان، بی خطر نیستند. اخیراً، مطالعاتی برای پیدا کردن مواد اسکولکس کش طبیعی با اثربخشی بیشتر و عوارض جانبی کم انجام شده است.

هدف: در این مطالعه اثر عصاره اتانولی زنجبیل بر روی پروتواسکولکس های کیست هیداتید بررسی شد.

روش کار: تعداد معینی از پروتواسکولکس های کیست هیداتید (حدود ۵۰ تا ۱۰۰) تحت مجاورت غلظت های مختلف عصاره اتانولی زنجبیل (۴۰، ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ mg/ml)، در دوره های مختلف زمانی (۱۵، ۳۰، ۴۵ و ۶۰ دقیقه) قرار گرفت. هر غلظت/ زمان سه مرحله انجام شد و پس از آن، زنده مانده پروتواسکولکس ها با رنگ آمیزی ائوزین ۱٪ بررسی گردید.

نتایج: میزان مرگ و میر با عصاره اتانولی زنجبیل پس از ۶۰ دقیقه به ترتیب ۶۸، ۹۲/۳، ۹۳ و ۱۰۰٪ در غلظت های ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ mg/ml بود و تفاوت معنی داری بین سه غلظت ۲۰۰، ۱۵۰ و ۱۰۰ وجود نداشت ($P > 0.05$). هر چند، در غلظت ۲۰۰ mg/ml پس از ۳۰ دقیقه انکوباسیون، ۱۰۰٪ پروتواسکولکس ها مرده بودند. بر اساس آزمون توکی، اختلاف معنی داری در درصد پروتواسکولکس های زنده بین غلظت های مختلف عصاره زنجبیل، به استثنای غلظت های ۱۵۰ و ۱۰۰ مشاهده نشد ($P > 0.05$).

نتیجه گیری نهایی: عصاره اتانولی زنجبیل دارای اثر اسکولکس کشی بالایی در محیط آزمایشگاهی است و می تواند به عنوان عامل اسکولکس کش در درمان جراحی کیست های هیداتید مورد استفاده قرار گیرد.

واژه های کلیدی:

عصاره اتانولی، زنجبیل، کیست هیداتید، بررسی آزمایشگاهی، اثر اسکولکس کشی