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ORIGINAL ARTICLE

# In vitro effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus protoscoleces*



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**Abstract** Hydatid disease is an important economic and human public health problem with a wide geographical distribution. Surgical excision remains the primary treatment and the only hope for complete cure of hydatosis. The most important complications arising from surgical excision, however, is recurrence, which is due to dissemination of protoscolices during the surgery. Pre-surgical inactivation of the contents of the hydatid cyst by injection of scolicidal agent into the cyst has been used as adjunct to surgery in order to overcome the risk of recurrence. In the present study, ethanolic extracts of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) were tested as scolicidal agent for *Echinococcus protoscoleces*. Protoscoleces were collected aseptically from sheep livers containing hydatid cysts. Three concentrations (10, 30 and 50 mg/ml) of each extract were investigated and viability of the protoscoleces was tested by 0.1% eosin staining. Ginger extract showed the strongest scolicidal effect (100%) after 20 min at a concentration of 30 mg/ml and 10 min at 50 mg/ml. The maximum scolicidal effect of turmeric was 93.2% after 30 min at a concentration of 50 mg/ml. It is concluded that turmeric and ginger extracts have high scolicidal activity and could be used as effective scolicidal agents against *Echinococcus protoscoleces*.

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## 1. Introduction

Echinococcosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*, is considered to be one of the most important global zoonotic diseases and widespread worldwide (Rajabloo et al., 2012; Pensel et al., 2014). The adult worms occur in the small intestine of dogs, and occasionally other

carnivores, while the larval stage can establish itself in a wide range of intermediate hosts, including cattle, sheep, pigs, horses and humans (Gholami et al., 2013). Infection of these hosts may occur after ingestion of infective eggs in contaminated food or water (Moazeni and Roozitalab, 2012). Infection is a result of the development of hydatid cysts, mainly in the liver and lungs, although cysts can arise anywhere in the body (Budke et al., 2009).

Surgical removal of the intact hydatid cyst remains the preferred method of therapy (Gholami et al., 2013) but surgery can increase the chance of intraoperative spillage of scolices, which is a major cause of recurrence and multiple secondary echinococcosis (Kilicoglu et al., 2008; Moro and Schantz, 2009). Many scolicidal agents have been used as an adjunct to surgery as a prophylactic means of preventing spillage of the contents of the cyst (Blanton et al., 1998; Spicher et al., 2008). The most frequently used agents are formalin, hypertonic saline, alcohol and povidone iodine (Karaoglanoglu et al., 2011). However, it has been reported that the use of these agents are accompanied in most cases with toxicity and severe hepatobiliary complications and also they may cause fatal hyperthermia (Yetim et al., 2005; Topcu et al., 2006; Adas et al., 2009; Karaoglanoglu et al., 2011).

Therefore, there is an urgent need for scolicidal agents that are less harmful to the patient, and also more effective for use in hydatid cyst surgery (Adas et al., 2009). Recently, efforts have been made to discover new anti-scolicidal compounds from sources such as plants and microorganisms (Moazeni et al., 2012). Zingiberaceae is one of the largest families in the plant Kingdom and is an important natural resource that offers several useful products including food, spices, medicines, dyes, perfume and aesthetics (Sirirugsa, 1999). Members of the Zingiberaceae family such as turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) have been used for many years as spices and in traditional forms of medicine to treat a variety of diseases (Flores-Sanchez and Gang, 2013). Both plants are rich in phyto-constituents such as alkaloids, saponins, flavonoids, terpenes and steroids, which are widely used as drug components in medicine (Singh et al., 2011). Since both plants have been shown to have a number of medicinal properties, the present study aimed to evaluate the *in vitro* scolicidal effects of the ethanolic extracts of both plants on the protoscolices of hydatid cysts.

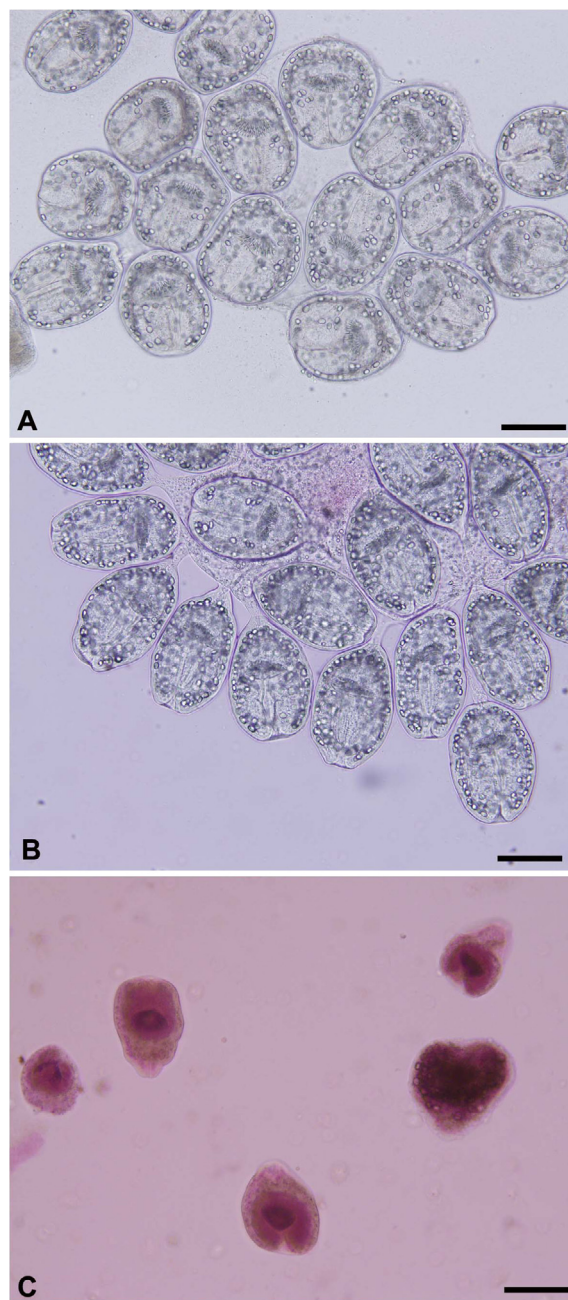
## 2. Materials and methods

### 2.1. Protoscolices collection

Hydatid cysts were collected from the liver of naturally infected sheep that had been slaughtered in a Riyadh abattoir, Saudi Arabia. The hydatid fluid was aseptically transferred into glass cylinders and left to set for 30 min to allow the protoscolices to settle to the bottom of the cylinders. The supernatant was then removed and the settled protoscolices were washed three times in normal saline. The viability of the protoscolices was confirmed by their motility characteristics and a 0.1% eosin staining test under light microscopy. Finally, the live protoscolices were transferred into a dark container containing normal saline and stored at 4 °C for further use.

### 2.2. Viability test

In the present study, the viability of the protoscolices was tested with 0.1% aqueous solution of eosin stain (1 g of eosin powder in 1000 ml distilled water). Five minutes after exposure to the stain, dead protoscolices appeared stained while the viable ones remained colourless under the microscope (Smyth and Barret, 1980). The red purple stained protoscolices were considered dead while unstained ones were recorded as alive (Fig. 1). The percentage of viability was determined by



**Figure 1** Live non stained protoscolices (A), live protoscolices after staining with 0.1% eosin (B), dead protoscolices after treatment with extracts and staining with 0.1% eosin (C). Scale-bar = 100  $\mu$ m.

counting a minimum of 450 and mostly more protoscolices and then calculating the number of viable protoscolices divided by the total number of protoscolices (Haghani et al., 2014). A percentage of viable protoscolices within the sediment of 95% or more, was considered to be appropriate for further experiments.

### 2.3. Preparation of extracts

Turmeric (*C. longa*) and ginger (*Z. officinale*) were collected from a local market in Riyadh city, Saudi Arabia. Powder totalling 500 g from each plant was extracted separately with 70% ethanol as follows: 100 g of dry powder was added to 400 ml of 70% ethanol and mixed gently for one hour using a magnetic stirrer. The obtained solution was left at room temperature for 24 h before being stirred again and filtered. The solvent was then removed by evaporation in a rotary evaporator. The alcohol free residue of each extract was weighed to give 5.86 g in the case of turmeric and 7.30 g in the case of ginger.

### 2.4. Determination of in vitro effects

In the present study, we tested three concentrations (10, 30 and 50 mg/ml) of both extracts for 10, 15 and 30 min on protoscolices. In order to prepare these different concentrations, respectively, 0.1, 0.3 and 0.5 g of dried extract was dissolved in 10 ml of distilled water. In each experiment, 2.5 ml of each concentration was placed in a test tube. Approximately  $5 \times 10^3$  protoscolices was then added to the tube and mixed gently, and the tube was then incubated at 37 °C for 10, 15 and 30 min. At the end of each incubation period, the upper portion of the solution was discarded, taking care to avoid disturbing the settled protoscolices. One millilitre of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. After 5 min, the upper portion of the solution was again discarded. The remaining settled protoscolices were smeared on a glass slide, covered with a cover glass and examined microscopically for viability. At least  $5 \times 10^3$  protoscolices in 2.5 ml distilled water with no exposure to either of the extracts formed a control group and each experiment was performed in triplicate.

### 2.5. Statistical analysis

One-way ANOVA was performed using a statistical package programme (Sigma Plot version 11.0). All *p* values are two-tailed, and  $P \leq 0.001$  was considered as significant.

## 3. Results

The scolicidal effects of different concentrations of *C. longa* and *Z. officinale* extracts are summarized in Tables 1 and 2.

Regarding *C. longa*, given a concentration of 10 mg/ml, mortality rates of 39.2%, 48.1% and 57.2% were observed following treatment periods of 10, 20 and 30 min, respectively. When the concentration of *C. longa* was increased to 30 mg/ml, mortality rates of 46.0%, 53.0% and 61.9% were observed at the same time intervals. A *C. longa* concentration of 50 mg/ml, meanwhile, led to mortality rates of 71.0%, 81.3%

**Table 1** Scolicidal effect of turmeric (*Curcuma longa*) extracts on the viability of protoscolices of *E. granulosus*.

Concentrations	Experiment	% of mortality rates after exposure		
		10 min	20 min	30 min
10 mg/ml	1	40.1	48.7	55.9
	2	39.6	46.3	58.8
	3	38	49.5	57.3
	Average	39.23333	48.16667	57.2
30 mg/ml	1	46.6	53.3	60.2
	2	47.2	55.3	63.5
	3	44.3	50.6	62.0
	Average	46.03333	53.06667	61.9
50 mg/ml	1	73.6	80	92.2
	2	70.2	81.9	93.4
	3	69.3	82.1	94
	Average	71.03333	81.33333	93.2
Control	1	8.1	12.6	19.1
	2	7.3	13.2	20.3
	3	7.5	11.8	19.6
	Average	7.6	12.5	19.6

**Table 2** Scolicidal effect of ginger (*Zingiber officinale*) extracts on the viability of protoscolices of *E. granulosus*.

Concentrations	Experiment	% of mortality rates after exposure		
		10 min	20 min	30 min
10 mg/ml	1	46.2	68.1	79.2
	2	48.4	66.9	78.3
	3	45.7	70.4	80.1
	Average	46.76667	68.46667	79.2
30 mg/ml	1	73.3	95.3	100
	2	76.1	93.3	100
	3	74.7	94.1	100
	Average	74.7	94.23333	100
50 mg/ml	1	94.2	100	100
	2	93.2	100	100
	3	90.8	100	100
	Average	92.73333	100	100
Control	1	8.1	12.6	19.1
	2	7.3	13.2	20.3
	3	7.5	11.8	19.6
	Average	7.6	12.5	19.6

and 93.2% after 10, 20 and 30 min, respectively. The mortality rates of protoscolices following exposure to *Z. officinale* extract at a concentration of 10 mg/ml were 46.7%, 68.4%, and 79.2% after 10, 20 and 30 min of application, while for a concentration of 30 mg/ml, the mortality rates were 74.7%, 94.3% and 100% at the same time intervals. The highest mortality rate, however, was observed at a concentration of 50 mg/ml, where treatment durations of 10, 20 and 30 min returned mortalities of 92.7%, 100% and 100%, respectively. Compared to the control group, the difference between the mortality rates due to effects of *C. longa* and *Z. officinale* extracts was statistically highly significant ( $P < 0.001$ ) for all three concentrations of both and at each of the various application times.

#### 4. Discussion

Surgical removal is the preferred method for the treatment of hydatid cysts (Gholami et al., 2013), and inactivation of the parasite with protoscolicidal agents is an important component of surgical treatment in order to avoid recurrence and multiple secondary echinococcosis (Haghani et al., 2014). Many protoscolicidal agents have been used, including hypertonic saline, alcohol, and povidone-iodine (Karaoglanoglu et al., 2011). However McManus et al. (2003) have argued that there is no ideal agent which is both effective and safe. It is extremely important, therefore, to identify an effective alternative protoscolicidal agent, especially to overcome the severe side-effects of the synthetic pharmaceuticals (Moazeni and Nazer (2011); Abdel-Baki et al., 2016). Since herbal extracts have been recognised as having the potential to be effective and safe alternative agents (Elissondo et al., 2008), the present study has sought to investigate the protoscolicidal effect of ethanolic extracts of turmeric and ginger for use against protoscolices in hydatid cysts.

The results proved that *C. longa* extract induced a significant scolicidal effect at all concentrations and exposure times. *C. longa* has long been used as a herbal medicine for different medical purposes (Maheshwari et al., 2006), and previous studies have revealed *C. longa* possess a multitude of beneficial effects in the treatment of cancers, cardiovascular disease and inflammation (Akram et al., 2010). *C. longa* extract has also been proven to have anti-parasitic activities against *Leishmania*, *Giardia lamblia*, *Trypanosoma* and *Schistosoma* (Morais et al., 2013). This study, however, is the first report that testifies the scolicidal activity of ethanolic extract of *C. longa*.

Regarding ginger (*Z. officinale*), it has been previously been established that ginger has anthelmintic activity against *Dirofilaria immitis*, *Anisakis simplex*, *Schistosoma mansoni* and *Hymenolepis nana* (Lina et al., 2014), while Moazeni and Nazer (2011) investigated the protoscolicidal activity of methanolic extracts of *Z. officinale*. They found that methanolic extract of *Z. officinale* had a 100% mortality rate at concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml after 60, 40 min and 30 min of exposure respectively. In the present study, however, we observed 100 % mortality rate at concentrations of 30 mg/ml and 50 mg/ml of ethanolic extract of *Z. officinale* after 30 min and 20 min of exposure respectively. This means that ethanolic extract of *Z. officinale* has a greater scolicidal effect in a shorter exposure time than the methanolic extract.

Our results strongly suggest, therefore, that ethanolic extracts of *C. longa* and *Z. officinale* have an anti-helminthic effect against protoscolices of hydatid cysts, with *Z. officinale* having a considerably greater effect than that observed with *C. longa*. Further in vivo and in vitro studies are needed to more fully evaluate the potential of these extracts, or some of their pure components, as useful alternatives for the treatment of hydatidosis.

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