

Research Article

PROTOSCOLICIDAL ACTIVITY OF THE CRUDE EXTRACT OF STEM BARK OF OAK (*Quercus* sp.)

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Abstract

The *Quercus* species (Oak) have been used for treating many human disorders since ancient time, and that is an encouragement to researchers to investigate the activity of Oak extract against more pathogenic diseases. *Echinococcus* sp. is a causative agent of an endemic parasite disease called Hydatidosis disease, in many countries. Using a safe and effective protoscolicidal agent during surgery for removing the *Echinococcus* cyst is an important step in completing treatment. The present study was designed to evaluate the activity of the ethanol extract from the stem bark of Oak against the protoscolices of sheep hydatid cysts by using 0.1 % Eosin dye. Three concentrations (50, 100 and 200 mg/ml) were used to estimate protoscolicidal activity at various exposure times (5, 10 and 15 minutes). Data analysis was implemented using SPSS software, and two-way ANOVA test. The strongest scolicial efficiency was recorded after 15 minutes of exposure at all used concentrations with a 44.03 %, 32.43 % and 0 % survival rates respectively. Meanwhile, 5 minutes of exposure showed the lowest activity with 76.96 %, 57.06 % and 11.86 % survival rates at used concentrations respectively. Also, it was found that the ethanol extract of *Quercus* sp. can destroy all protoscolices after 10 minutes of exposure at a 200 mg/ml concentration. The obtained outcomes confirmed the scolicial efficacy of *Quercus* sp. ethanol extract with a short exposure *in vitro* as a natural scolicial agent.

Article History

Received : 10.07.2020

Revised : 23.07.2020

Accepted: 26.08.2020

Keywords: Scolicial agent, *Quercus*, Ethanol extract and Hydatidosis

1. Introduction

The *Quercus* genus is a woody angiosperm belonging to the Fagaceae family, and commonly called Gall Oak, Manjakani, Majuphal or Machakai (Nixon, 2006; Nik Mat Zin *et al.*, 2019). This genus has economic and ecological value, and it is widely distributed worldwide in the New World, Asia, Europe and North Africa (Nixon, 2006; Taib *et al.*, 2020). All parts of the *Quercus* species have been used in traditional medicine to cure many diseases including astringent, hemostatic effects and diarrhea, indigestion,

stomach pain, chronic skin diseases, inflammations of the genitals, vaginal discharge and asthma (Jamal *et al.*, 2011; Nik Mat Zin *et al.*, 2019; Taib *et al.*, 2020). Pharmacological studies have confirmed various effects of the *Quercus* species, such as cytotoxic, anticancer, antimicrobial, anti-inflammatory, neuroprotective, antidiabetic, antioxidant, antiobesity, hepatoprotective and gastrointestinal as well as positive effects on skin disorders (Jain, 2019; Taib *et al.*, 2020). Nik Mat Zi Net al. (2019) reviewed the activity of *Quercus infectoria* against parasites amoebiasis, blastocystis and leishmanial, and they suggested that more research should be carried out on the activity of *Quercus* against parasites

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Echinococcus species are tapeworm parasites, belong to the family Taeniidae. Adult tapeworm of the *Echinococcus* sp. occupy the intestines of definitive hosts such as dogs and foxes; this adult stage produces taeniid eggs which pass with the feces of the definitive host (Torgerson, 2014). After ingestion of eggs by the intermediate hosts, the eggs develop into metacestode, and protoscolices, then form a cyst which fills with fluid in one or more organs of the host, most commonly the liver or lung. The formation of cystic Echinococcosis in humans and other mammals is called Hydatidosis or Echinococcosis (CE) disease (Arandes and Bertomeu, 2012). This disease is a health issue in many countries including Africa, South America and the Middle East. It causes significant economic damage due to decreased production of animal products such as meat and milk, and decreased fertility, besides various human symptoms of CE which can be complicated because of cystic rupture and bacterial infection (Torgerson, 2014; Pavletic *et al.*, 2017). Among the different ways to treat CE, surgery with protoscolicide is one of the best methods. However, spillage of the parasite from the primary cyst and bacterial infection are significant risks during surgery. Therefore, the use of an effective scolicedal agent reduces the risk of the formation of secondary Echinococcosis (Arandes and Bertomeu, 2012). Due to the toxicity and insufficiency of common scolicedal agents like silver nitrate, hypertonic sodium chloride and cetrimide, exploring natural scolicedal agents has attracted the attention of many researchers in an attempt to find a more effective agent (Caglar *et al.*, 2008; Barzim *et al.*, 2019). Accordingly, this study aims to evaluate the scolicedal activity of Oak ethanol extract against protoscolices collected from the liver of infected sheep at different exposure times.

2. Materials and Methods

Plant Materials

The bark material of *Quercus* sp. (Oak) was obtained from the local market in Basrah city, Iraq, and it was identified and confirmed by a botanist in the biology department, College of Science, University of Basrah. The plant material

was ground into a fine powder using an electric mill. Then, the plant powder was saved in dark glass containers until it was used in subsequent experiments.

Extract Preparation

Plant extract was prepared by using a ratio 10:1 of ethanol (90 %) to plant material (g). The mixture was shaken with a magnetic stirrer for 8 hours, then left for 48 hours to soak at room temperature. The solution was filtered using a filter paper (Whatman No.1), and a rotary evaporator under vacuum; the extra solvent was removed.

Collection and Viability of the Protoscolices

Fresh livers of naturally infected sheep with *Echinococcus granulosus* hydatid cysts were obtained from slaughtered animals at the Basrah Governmental Abattoir. The hydatid cyst fluid and protoscolices were aspirated using a sterile (5 ml) syringe and collected into a sterile (50 ml) Falcon tube. The protoscolices were washed three times with a phosphate buffered saline PBS (pH 7.2) then centrifuged at 1000 g for 4 minutes. The protoscolices were allowed to precipitate, then the supernatant was removed. The viability of the protoscolices was examined by adding 0.1 % of Eosin solution to the protoscolices solution in a ratio of 1:1. The change of color under a light microscope was noticed; the dead protoscolices were colored and the living protoscolices remained colorless. The protoscolices suspension was considered suitable for use when > 95 % of the protoscolices were viable (Mahmoudvand, 2016).

Scolicedal Assay

Our study examined the effect of different concentrations (50, 100 and 200 mg/ml) of the *Quercus* sp. extract against *Echinococcus granulosus* protoscolices for 5, 10 and 15 min. 1 ml of the protoscolices solution (containing approximately 500 protoscolices/ml) was placed in each test tube and 1 ml of the ethanol extract was added in different concentrations to each test tube. The tubes were incubated for 5, 10, 20 and 30 min at 37 °C, then the supernatant was

removed at the end of the incubation period. A volume of 1 ml of 0.1% eosin stain was added to the remained protoscolices and mixed gently, smeared on a glass slide, covered with a coverslip and examined under a light microscope. The percentage of dead protoscolices was counted and non secilocsotorp detaert were used for controls. The experiments were repeated three times (Abdel-Baki *et al.*, 2016).

Statistical Analysis

Statistical analysis was undertaken using SPSS software version 24. To identify the differences between the control group and the test a two-way ANOVA test was utilized, and a value of less than 0.5 was considered significant.

3. Results and Discussion

The ethanol extract of *Quercus* sp. showed significant protoscolicidal activity in comparison with the control group ($P < 0.05$) at all exposure times and in all used concentrations (Table - 1). Also, the survival rate decreased as the time of exposure and concentration increased (Figure - 1). The highest scolicial effect (0 % survival rate) was recorded at a concentration of 200 mg/ml and after 10 minutes of exposure time in this study. Galehdar *et al.* (2018) found a similar scolicial activity for *Nectaros cordumkoelzi* methanol extract (100 % mortality rate) but at a higher concentration (500 mg/ml) and with the same exposure time (10 min) while *Nigella sativa* oil exhibited a 100 % mortality rate at a lower concentration (100 mg/ml) and long exposure time (120) (El-Bahy *et al.*, 2019).

In the present study, 50 mg/ml of 90 % ethanol extract of *Quercus* sp. gave a survival rate of 44.03 % after 15 minutes of exposure time. Meanwhile, the application of methanol extract of *Quercus infectoria* Olivier gall, and the 70 % ethanol root extract of *Salvadora persic* resulted in 0 % survival rates at the same concentration (50 mg/ml) but with more exposure time (20 min) (Abdel-Baki *et al.*, 2016; Malekifard and Keramati, 2018).

Phenols, Flavonoids, Terpenes and Tannin compounds have been identified from *Quercus* species (Jain, 2019). Endo *et al.* (2019) connected the anti-toxoplasma activity of the bark of *Quercus cris pula* to the pentacyclic triterpenoid compounds known as ursolic acid acetate, oleanolic acid acetate and 29-norlupane-3,20-dione. The activity and selectivity of these compounds was associated with more polarity functional group, such as the hydroxyl group or carboxyl on E-ring and the modification of the 3-hydroxy group in the case of oxidation to carbonyl or acetylation (Endo *et al.*, 2019). Numerous compounds have been isolated from *Quercus* cortex, and have shown antibacterial and anti-quorum sensing (QS) activities. The most active ones are 4-propyl-1,3-benzenediol, 1,2,3-trihydroxybenzene, 1,2,3-trihydroxybenzene (pyrogallol), 4-(3-hydroxy-1-propenyl)-2-methoxy-phenol benzenediol (coniferyl alcohol) 2H-1-benzopyran-2-one (coumarin) and its derivative 7-hydroxy-6-methoxy-2H-1-benzopyran-2-one (scopoletin) (Deryabin and Tolmacheva, 2015).

The mechanism of the action of Oak extract could be due to the effect of compounds on the protease action of the parasite, as De Pablos *et al.* (2010) revealed that maslinic acid acted as a protease inhibitor against the *Toxoplasma gondii* parasite that is necessary for cell invasion. 2-deoxy analog of maslinic acid, namely Oleanolic acid, has been isolated from *Quercus crispula* (Endo *et al.*, 2019). Further, it has been suggested that the action of Oak extract could be due to clotting of cytoplasm, leaking of cell substances and demolition of cytoplasmic membrane proteins (Jain, 2019).

Table - 1: Scolicidal activities of different concentrations of Ethanol *Quercus* sp. Bark extract at different exposure times

| Concentration Mg/ml | Survival rate | | | Mean of survival rate in concentrations | Comparison groups |
|--|---------------|---------|---------|---|----------------------|
| | 5 min | 10 min | 15 min | | |
| 0 | 100 % | 100 % | 100 % | 100 | $p>0.05$ |
| 50 | 76.96 % | 63.33 % | 44.03 % | 61.44 | $p<0.05$ |
| 100 | 57.06 % | 45.50 % | 32.43 % | 45.00 | $p<0.05$ |
| 200 | 11.86 % | 0.00 % | 0.00 % | 3.95 | $p<0.05$ |
| Mean of survival rate in exposure times | 61.47 | 52.20 | 52.20 | | |

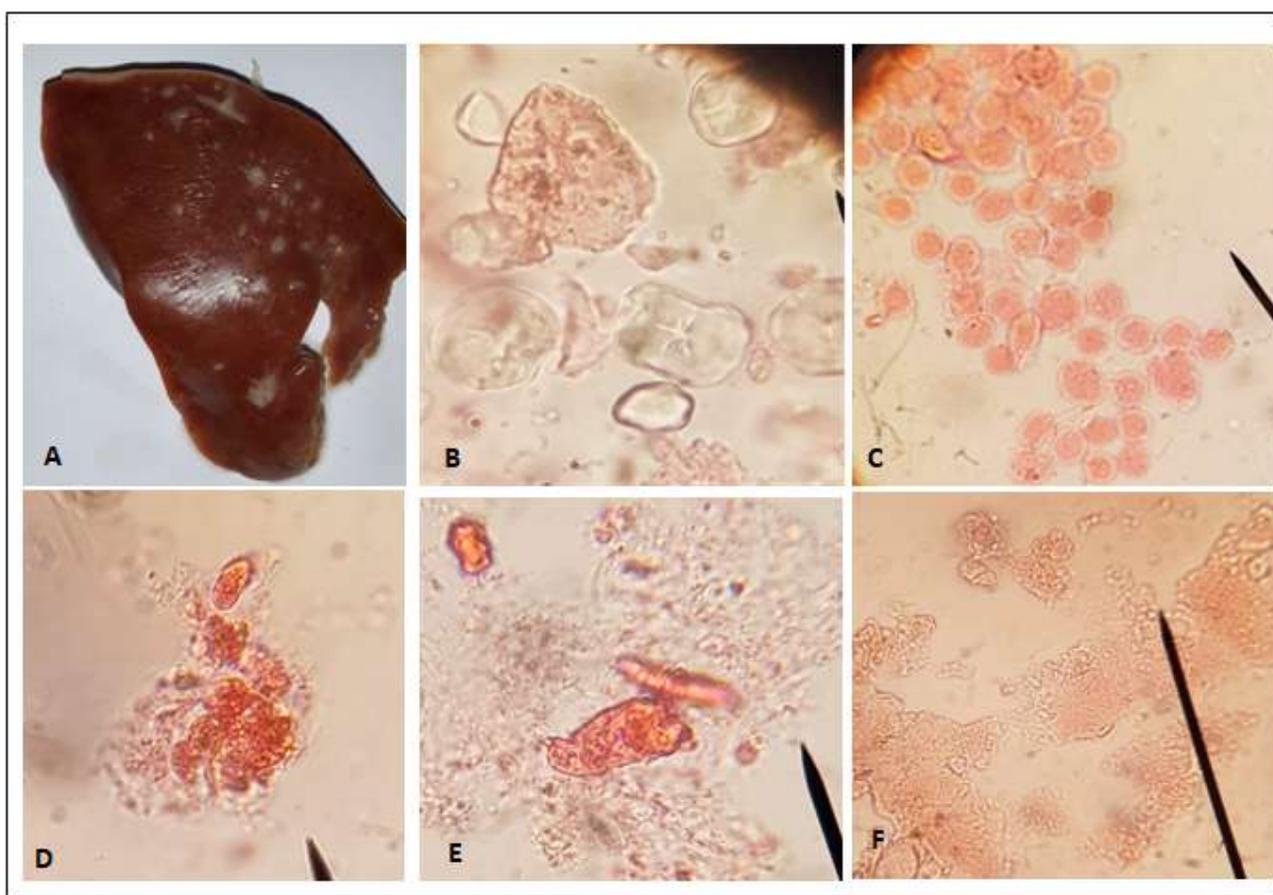


Figure - 1: A- Sheep livers with hydatid cysts; B - Protoscolices of hydatid cyst before dying; C - Viable protoscolices after using 0.1% of eosin dye; D - Dead protoscolices after effect of 50 mg/ml ethanol extract of *Quercus* sp. and 0.1% of eosin; E- Dead protoscolic

4. Conclusion

In particular, ethanol extract of bark *Quercus* sp. extract exhibited high scolical activity against the protoscolices of *Echinococcus* in low concentrations and over short periods of exposure; in addition, it is easy to prepare for an in vitro model. However, an animal model of activity of Oak extract still needs to be assessed against hydatid infection.

5. References

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DOI Number

[DOI: 10.22192/Isa.2020.6.5.5](https://doi.org/10.22192/Isa.2020.6.5.5)

Thomson Reuters Researcher ID

L – 5547 – 2016

ISI Impact Factor

4.206

How to Cite this Article:

Areej Hasan S. Aldhafer and Shaimaa A. Alsamir. 2020. Protoscolicidal activity of the Crude extract of Stem Bark of Oak (*Quercus* sp.). *Life Science Archives*, 6(5): 1969 – 1974.

[DOI: 10.22192/Isa.2020.6.5.5](https://doi.org/10.22192/Isa.2020.6.5.5)