*Surgical Science*, 2012, 3, 452-456
doi:10.4236/ss.2012.39089 Published Online September 2012 (http://www.SciRP.org/journal/ss)

# Sumac (*Rhus coriaria* L.): Scolicidal Activity on Hydatid Cyst Protoscolices

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Received May 4, 2012; revised June 10, 2012; accepted June 30, 2012

## ABSTRACT

**Background:** Few anthelmintics are available for the treatment of hydatid disease caused by the parasite *Echinococcus granulosus.* The appearance of resistance to synthetic anthelmintics and the adverse side effects of chemical drugs, stimulated the research of alternatives, such as medicinal plants. In the present study, the scolicidal effect of methanolic extract of sumac (*Rhus coriaria*) was investigated. **Methods:** Protoscolices were aseptically collected from sheep livers containing hydatid cysts. Three concentrations of sumac extract (10, 30 and 50 mg/mL) were used for 10, 20 and 30 min. Viability of protoscolices was confirmed by 0.1% eosin staining. **Results:** While the rate of dead protoscolices was 16.93% in the ontrol group, when protoscolices were exposed to sumac extract at the concentration of 10 mg/mL , the rate of dead protoscolices increased to 94.13%, 97.67% and 100% after 10, 20 and 30 minutes, respectively. The mortality rate of protoscolices increased to 98.89%, and 100% when they were exposed to 30 mg/mL concentration of sumac extract for 10 and 20 minutes respectively. One hundred percent mortality rate was observed at concentration of 50 mg/mL after 10 min of exposure. **Conclusions:** This *in vitro* study showed that methanolic extract of *R. coriaria* may be considered as an effective natural scolicidal agent.

**Keywords:** Hydatid Cyst; Scolicidal; Methanolic Extract; Sumac; *Rhus coriaria*

## 1. Introduction

Many tapeworms alternate their developmental cycle between intestinal stages in one host and tissue stages in another. Hydatid disease is the result of tissue invasion with the intermediate stage of a dog tapeworm, *Echinococcus granulosus*. The adult stage is a largely innocuous small tapeworm of dogs and other canids. The invasive intermediate stage (metacestode) takes the form of an enlarging cyst primarily in the liver and lungs of domestic and wild herd animals. These cysts may be found singly, in clusters, or in such numbers that they pack the peritoneal cavity. The principal sources of morbidity are pressure effects from cyst size (up to 48 liters), location in a sensitive organ (brain, reproductive tract, bone), or cyst rupture with subsequent anaphylaxis or dissemination of the infection. The disease can be found in any part of the world where slaughtering practices allow dogs to consume the organs of infected animals. The parasite can then complete its developmental cycle [1,2]. There are currently three treatment options for hydatid disease, surgery, ultrasound-guided aspiration, and chemotherapy [3]. Each of these modalities has limitations depending on the specific case. Chemotherapy is the preferred treatment where, surgeons are not available or the cysts are too numerous, and in inoperable cases, chemotherapy is the only option. Chemotherapy has also been used as an adjunct to surgery for prophylaxis against spillage of cyst contents [3,4]. For treatment of human hydatid disease, the best agent available is the benzimidazole albendazole. Most studies indicate that the efficacy of albendazole as measured by the disappearance of a cyst is generally less than 30% under ideal circumstances. Altogether, 60% of cysts show some response in the course of therapy, including shrinkage in size or detachment of cyst components from the wall. Albendazole must be taken daily for 4 to 6 weeks, and this course should be repeated an additional two or three times. The poor response of this infection to most chemotherapeutic agents has made hydatidosis primarily a surgical disease, and thus the role of chemotherapy is for prophylaxis against spillage during surgery, for the treatment of inoperable cases, or for use in areas without adequate surgical facilities. There is clearly a need for drugs that are more effective and easier to administer [2]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as plants, animals and microorganisms. Recently, herbal medicines have increasingly been used to treat many diseases including several infections [5].

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Sumac (*Rhus coriaria* L., family Anacardiaceae) grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and the Southeastern Anatolian Region of Turkey. The name is derived from "sumâqâ", meaning red in Syriac. The spice, produced by grinding the dried fruit with salt, is used as a condiment and sprinkled over kebabs and grilled meat as well as over salads that often accompany these dishes. It has a sour taste (pH 2.5) which is derived from the citric and malic acids found in its juice [6]. Sumac is widely used in Turkey and the Middle East, the fruits are red colored and contain one seed. However, this part of the plant is typically consumed as spice after drying and grinding [7]. Sumac has been known to possess dietary and medicinal properties. In folk medicine, it is used for treatment of indigestion, anorexia, diarrhea, hemorrhagia and hyperglycemia [8]. Antioxidant [8-14], antidiabetic [15], hypo glycemic [16], antifibrogenic [17] and antitumorigenic [18, 19] properties of Sumac have been previously addressed. It also has been shown to be useful in the treatment of osteoarthritis [20].

The present study was conducted to evaluate the in vitro scolicidal effect of methanolic extract of Sumac (*Rhus coriaria*) on the protoscolices of hydatid cysts.

## 2. Materials and Methods

### 2.1. Collection of Protoscolices

Hydatid cysts from livers of naturally infected sheep were obtained from Shiraz abattoir in southern Iran. The hydatid fluid was aseptically transferred into glass cylinders and left to set for 30 min. The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the yielded protoscolices were washed three times with normal saline. Viability was assessed by muscular movements and 0.1% eosin staining test. The live protoscolices were finally transferred into a dark container containing normal saline solution and stored at 4˚C for further use.

### 2.2. Preparation of Sumac Extract

Dried fruits of *R. Coriaria* (*Sumac*) were powdered mechanically using a commercial electrical blender. To obtain the methanolic extract, 100 g of dry *Sumac* powder was added to 400 mL of pure methanol and mixed gently for 1 h using a magnetic stirrer. The obtained solution was left at room temperature for 24 h. The solution was stirred again and filtered and then the solvent was removed by evaporation in a rotating evaporator. The remaining semisolid material was then freeze-dried. The obtained residue (6.2 g) was placed into a sterile glass container and stored at 4˚C for further use.

### 2.3. Scolicidal Assay

In this study, three concentrations of sumac extract (10, 30 and 50 mg/mL) were used for 10, 20 and 30 min. To prepare the sumac extract solution at 10, 30 and 50 mg/mL concentrations, 0.1, 0.3 and 0.5 g of dried extract was dissolved in 10 ml of distilled water, respectively. Then 2.5 mL of each sumac solution was placed in test tubes, to which a drop of protoscolex-rich sediment was added. The contents of the tubes were gently mixed .The tubes were then incubated at 37˚C for 10, 20 and 30 min. At the end of each incubation time the upper phase was carefully removed so as not to disturb the protoscolices. One milliliter of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of protoscolices was then smeared on a manually scaled glass slide, covered with a cover glass (24 × 50 mm), and examined under a light microscope. The percentages of dead protoscolices were determined by counting a minimum of 500 protoscolices. Non treated protoscolices were considered as a control group in each experiment. The experiments were performed in triplicate.

### 2.4. Viability Test

In the present study, eosin stain with the concentration of 0.1% (1 g of eosin powder in 1000 mL distilled water) was used to check the viability of the protoscolices [21]. Fifteen minutes after exposure to the stain, the protoscolices with no absorbed dye were considered potentially viable (**Figure 1**), otherwise they were recorded as dead (**Figure 2**).

### 2.5. Statistical Analysis

Differences between the test and control groups were analyzed with Chi-square test. Statistical analysis was

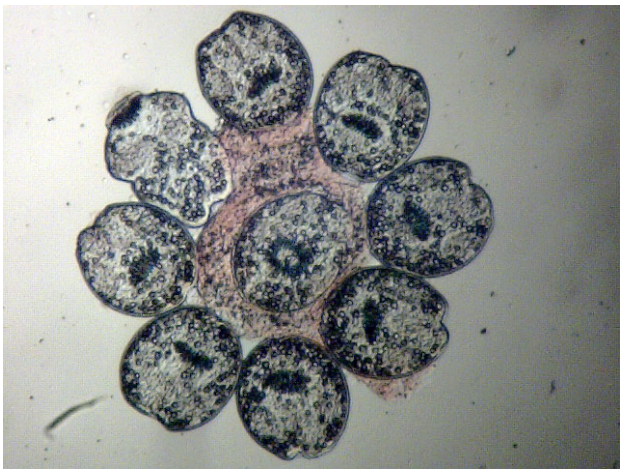

**Figure 1. Live protoscolices after staining with 0.1% eosin.**

 

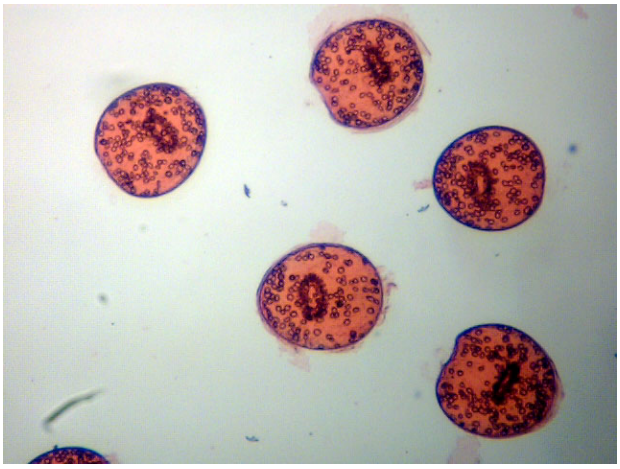

**Figure 2. Dead protoscolices after exposure to methanolic extract of *Rhus coriaria* and staining with 0.1% eosin.**

performed with GraphPad InStat software. P values less than 0.01 were considered to be significant.

## 3. Results

The mortality rate of hydatid cyst protoscolices after exposure to different concentrations of the methanolic extract of *R. coriaria* following various exposure times are presented in **Tables 1-3**. *Rhus coriaria* showed high scolicidal activity and its methanolic extract was found to be effective against protoscolices at all three concentrations tested. While the mortality rate of protoscolices was 16.93% in the ontrol group, when protoscolices were exposed to the *R. coriaria* extract at concentration of 10 mg/mL, the mortality rate increased to 94.13%, 97.67% and 100% after 10, 20 and 30 minutes, respectively. The mortality rate of hydatid cyst protoscolices after exposure to concentration of 30 mg/mL of *R. coriaria* extract was 98.89%, and 100% after 10 and 20 minutes respectively. One hundred percent mortality rate was observed with *R. coriaria* extract at concentration of 50 mg/mL after 10 min of exposure. The difference between the scolicidal effect of *R. coriaria* extract was statistically highly significant ($P < 0.0001$) for all three concentrations and at various exposure times, comparing to the control group.

**Table 1. Scolicidal effect of *Rhus coriaria* extract at the concentration of 10 mg/mL following various exposure times.**

| Exposure time (min) | Experiments | Protoscolices | Dead protoscolices | Mortality rate (%) |
|---|---|---|---|---|
| **10** | 1 | 789 | 727 | 92.14 |
| | 2 | 964 | 912 | 94.60 |
| | 3 | 753 | 720 | 95.61 |
| | **Total** | **2506** | **2359** | **94.13** |
| **20** | 1 | 866 | 845 | 97.57 |
| | 2 | 1341 | 1215 | 98.06 |
| | 3 | 800 | 777 | 97.12 |
| | **Total** | **3007** | **2937** | **97.67** |
| **30** | 1 | 586 | 586 | 100 |
| | 2 | 880 | 880 | 100 |
| | 3 | 963 | 963 | 100 |
| | **Total** | **2429** | **2429** | **100** |
| **Control** | | **2480** | **420** | **16.93** |

**Table 2. Scolicidal effect of *Rhus coriaria* extract at the concentration of 30 mg/mL following various exposure times.**

| Exposure time (min) | Experiments | Protoscolices | Dead protoscolices | Mortality rate (%) |
|---|---|---|---|---|
| **10** | 1 | 1038 | 1018 | 98.07 |
| | 2 | 1053 | 1051 | 99.81 |
| | 3 | 624 | 616 | 98.71 |
| | **Total** | **2715** | **2685** | **98.89** |
| **20** | 1 | 675 | 675 | 100 |
| | 2 | 1320 | 1320 | 100 |
| | 3 | 752 | 752 | 100 |
| | **Total** | **2747** | **2747** | **100** |
| **Control** | | **2480** | **420** | **16.93** |

**Table 3. Scolicidal effect of *Rhus coriaria* extract at the concentration of 50 mg/mL following various exposure times.**

| Exposure time (min) | Experiments | Protoscolices | Dead protoscolices | Mortality rate (%) |
|---|---|---|---|---|
| **10** | 1 | 506 | 506 | 100 |
| | 2 | 873 | 873 | 100 |
| | 3 | 637 | 637 | 100 |
| | **Total** | **2050** | **2050** | **100** |
| **Control** | | **2480** | **2480** | **16.93** |

## 4. Discussion

Few chemotherapeutic agents are available for the medical management of hydatid disease caused by the parasite *Echinococcus granulosus* [2]. The control of helminthosis, and, generally of all parasitic diseases is usually made with synthetic anthelmintics. Up to date, many chemical scolicidal agents have been used for inactivation of the hydatid cyst protoscolices. Many of these scolicidal agents may cause undesirable complications that limit their use. For example adverse side effects has been reported for 20% hypertonic saline, 20% silver nitrate, 0.5% - 1% cetrimide, ethyl alcohol, and 20 mg/mL albendazole sulfoxide [22]. The appearance of resistance to synthetic anthelmintics stimulated the research of alternatives, such as medicinal plants [23]. According to circumstances and depending on their efficacy, naturally produced plant anthelmintics offer an alternative that can overcome some of these problems and is both sustainable and environmentally acceptable [24]. Among the most promising advances in the field of drug development is discovering new molecules or novel uses of the already available compounds with known safety and without any side effects [25]. A number of studies describe the inhibitory effects of different herbs and spices and their volatile components on a variety of microorganisms. For

 

instance, sumac has been shown to possess antimicrobial [26,27], antibacterial [6,28-31], antiviral [32], antimalarial [33] and antifungal [34] properties.

In the present study we investigated the potency of methanolic extract of sumac (*R. coriaria*) on the protoscolices of hydatid cyst. The results of our study showed that sumac extract has a high scolicidal activity at the concentrations of 10, 30 and 50 mg/mL after 30, 20 and 10 min of application, respectively. In previous study, we investigated the protoscolicidal activity of garlic (*Allium sativum*). Methanolic extract of garlic had 100% scolicidal effect at a concentration of 25 mg/mL after 60 min of exposure [23]. In the present study we observed a higher scolicidal effect (100%) with methanolic extract of sumac at a lower concentration (10 mg/mL) and in a shorter exposure time (30 min).

Very limited literature is available on the mechanism for the antimicrobial activity of herbs and spices 6. As for the compounds in sumac which may be responsible for the antimicrobial activity, more than 120 volatile constituents have been identified by using gas chromatography and mass spectroscopy, of which terpenoids and aliphatic compounds were found occurring more frequently in six different varieties of sumac. Main constituents of *R. coriaria* are terpene hydrocarbons (*i.e.* $\alpha$-pinene, $\beta$-caryophyllene and cembrene), oxygenated terpenes (*i.e.* $\alpha$-terpineol, carvacrol and $\beta$-caryophyllene alcohol) as well as farnesyl acetone, hexahydrofarnesyl acetone and aliphatic aldehydes [35].

This *in vitro* study showed that methanolic extract of *R. coriaria* is an effective scolicidal agent. To the best of our knowledge, this is the first report that investigates the scolicidal efficacy of sumac extract on the protoscolices of hydatid cysts. The results of this study allowed us to suggest that sumac (*R. coriaria*) is likely source of new compounds that could be used as an effective scolicidal agent. Further studies will be necessary to identify and isolate these active compounds. The results of present study open the possibility of more investigations of *in vivo* scolicidal effect of this traditional medicine.

## 5. Acknowledgements

The authors received financial support from Shiraz University (grant No. 87-GR-VT-24). We acknowledge Mr. A. Motabi Alavi for his kind assistance during this study.

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