

# Effect Of Ethanol Extract Of Capsicum sp. On The Protoscolices Of Sheep Hydatid Cysts And Pathogenic Bacteria

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**ABSTRACT** -Background: Hydatidosis is a zoonotic disease that requires surgery as one of the best methods for treatment, in conjunction with scolical agents to prevent the formation of new cystic echinococcosis. Bacterial infection is another risk in hydatidosis surgery. Due to the side effects of chemical scolical agents, this study aimed to evaluate the effect of ethanol extract of *Capsicum* sp. on the protoscolices of hydatid cysts and some pathogenic bacteria species *in vitro*.

Methods: The hydatid cysts of *Echinococcus* sp. were collected from the livers of infected sheep. The scolical effect was determined at different exposure times (5, 10, 15 mins.) and different concentrations (50, 100, 500 mg/ml), and the viability of the protoscolices was detected by 0.1 % eosin staining. The antibacterial activity of the Capsicum ethanol extract was estimated using the agar well diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The secondary metabolites of ethanol extract were assessed using several qualitative tests.

Results: The results showed the highest scolical efficacy after 15 mins of exposure at all used concentrations with a 22.4%, 19.8% and 0% survival rate respectively. The highest antibacterial activity was recorded against *Staphylococcus aureus* with MIC 125 mg/ml.

Conclusion: Capsicum ethanol extract has a good protoscolical effect in a short exposure time and could be used as a scolical agent. However, *in vivo* studies are necessary to confirm the effect.

**KEY WORDS:** Antibacterial: Capsicum: Ethanol extract: Hydatid cyst: scolical agents:

## 1. INTRODUCTION

Hydatidosis is a zoonotic parasitic disease in people and domestic animals that causes huge economic damage. Hydatidosis is caused by the larval stage of the *Echinococcus* species, Taeniidae family.<sup>1</sup> Dogs are the final host for the mature stage of *Echinococcus* spp., while humans and sheep are intermediate hosts infected by the ingestion of eggs.<sup>2</sup> The embryophore releases the oncosphere in the stomach, where the enzymatic action and bile assist the oncosphere to penetrate the wall and move to the liver, lungs and sometimes other internal organs to stay and cause Cyst Echinococcus (CE).<sup>3</sup>

The best method for treating CE is surgery with the injection of protoscolical agents into the hydatid cyst to avoid the risk of spillage of the protoscolices and formation of a secondary cyst.<sup>4</sup> In addition, bacterial

infection of the hydatid cyst can occur and cause destruction of the endocyst and sterilization of the cyst which leads to many hazardous consequences.<sup>5-7</sup>

Many protoscolicidal agents have been used such as silver nitrate, formalin and hypertonic saline to sterilize cysts. However, the use of each agent involves risks of complication such as cholangitis and necrosis. Therefore, it has been suggested that further studies be conducted to find more effective and safer protoscolicidal agents.<sup>8-9</sup>

Medicinal plants have been widely used for over a thousand years to prevent or cure diseases.<sup>10</sup> Recently data have demonstrated that about third of medicines are derived from natural products including plants. Natural products from plants have played an important role in drug discovery as a direct source of medicine, or provide the raw material for the development of semi-synthetic new drugs or as lead molecules. Therefore, evaluation of medicinal plants is an aim in different countries.<sup>10-11</sup>

Capsicum or pepper (red, green, bell and chili) is a part of the Solanaceae family, *Capsicum* genus. In total, 5 out of 40 *Capsicum* species are consumed by humans as a flavoring, spice or vegetable added to food as a raw or cooked ingredient throughout the world.<sup>12</sup> Capsicum fruit is also used in folk medicine to reduce blood pressure, increase the efficacy of the circulatory system, break down cholesterol, and aid digestion.<sup>13</sup> In addition, it has been used to treat dysentery, diarrhea and stomach ache, toothache, asthma, ulcers, arthritis and for wound healing.<sup>12-14</sup> Many species of *Capsicum* have been documented as possessing antimicrobial properties against fungi and Gram positive and negative bacteria.<sup>15-16</sup> Dogan *et al.* determined that the methanol extract of *Capsicum annuum* was effective on *Helicabacter pylori* and *Campylobacter jejuni*, while its water extract was highly effective on *Arcobacter cryaerophilus*.<sup>17</sup> Moreover, red Capsicum has displayed effects on metabolic syndrome including obesity, diabetes and lipid mass, and revealed anticarcinogenic, antioxidant and antigenotoxic effects.<sup>18-19</sup> A few pieces of research in the literature have reported the anti-parasitic activity of the *Capsicum* species. Extracts of *C. frutescens* showed a potential effect against the fish ectoparasite *Ichthyophthirius multifiliis* and anthelmintic activity.<sup>18, 20</sup> Therefore, the aim of this study was to assay the protoscolicidal effects of ethanol extracts of the fruit of *Capsicum sp.* on the protoscolices of sheep hydatid cysts, which has not been studied before, and to investigate its antibacterial activities against two types of pathogenic bacteria.

## **2. MATERIALS AND METHODS**

### **2.1.Plant Materials:**

Fruits of the hot red pepper *Capsicum sp.* were purchased from a local market, dried, ground and extracted using 70% ethanol and a magnetic stirrer for 48 hours each. The extracts were filtered and the solvent was then removed under vacuum using a rotary evaporator.

### **2.2.Phytochemical Analysis:**

Ethanol extract of *Capsicum* was subjected to further analysis to detect the secondary metabolites. 1 g of each plant extract was dissolved in 20 ml of distilled water and filtrated; 1 ml of each filtrate extract was used for the phytochemical tests. The presence of alkaloids was detected using Dragendorff's reagent, flavonoids using alcoholic potassium hydroxide reagent, free amino groups using Ninhydrin, glycosides using Benedict reagent, phenols using ferric chloride and Folin reagent, saponins using mercury chloride reagent and tannins using lead acetate 1% reagent.<sup>21-24</sup>

### 2.3. Protoscolices Collection:

Hydatid cysts were sourced from the livers of infected sheep. The cysts were transferred to the laboratories and their surfaces were sterilized with outer 70% ethanol before being dissected. The cyst fluid was collected with a 5ml syringe and transferred into a sterile container, The supernatant was removed and the sediment protoscolices were washed three times with PBS.<sup>25</sup>

### 2.4. Viability of the Protoscolices:

This test was accomplished by adding 0.1% aqueous eosin to the protoscolices in a clean glass slide which was examined under a light microscope. The green protoscolices were considered to be alive and the red ones were considered to be dead.<sup>26</sup>

### 2.5. Scolicidal Assay

In this study, 1 ml of three concentrations (50, 100, 500 mg/ml) of ethanol extract of Capsicum, was placed in a test tube. Protoscolices sediment was added ( $\approx$  500 protoscolices/ml) and gently mixed. The test tubes were incubated for 5, 10 and 15 min at 37 °C for each concentration. Then the supernatant of the solution was carefully removed, and one millilitre of 0.1% eosin stain was added and mixed. The yielded protoscolices were then washed and examined under light microscope. For each experiment, minimum of 500 protoscolices were counted, and untreated protoscolices-on were designed as control groups. The experiments were repeated three times. Statistical analysis was carried out using SPSS software version 24. A two-way ANOVA test was used to compare each experiment. The least significant difference (LSD) was used to compare the mean survival rate of protoscolices for all used concentrations, exposure times and the control. A *P value* of less than 0.05 was considered significant.

### 2.6. Bacterial Isolates

Bacterial isolates were obtained from burned patients and identified in the microbiological lab at the Department of Biology, College of Science, University of Basrah, using traditional biochemical tests which were then confirmed by automated vitek<sub>2</sub> compact system.

### 2.7. Antibacterial Activity and Minimal Inhibitory Concentration Assay

This assay was performed using the agar well diffusion method as described in <sup>27</sup>. Wells were formed with a sterilized Pasteur pipette in a nutrient agar plate inoculated with tested bacteria. Bacterial concentrations were adjusted according to McFarland 0.5 standard tube and the final bacterial concentrations were streaked on a solid culture medium using sterilized cotton swabs. After the plates had been dried for 15-20 mins, wells were filled with 100  $\mu$ l of ethanol extract of Capsicum that was dissolved in dimethyl sulphoxide (DMSO), whereas the extract was prepared with serial dilutions (250, 125, 62.5 mg/ml) to determine MIC. The plates were incubated at 37 °C for 24 hours to form zones of inhibition through the agar media. The diameters of the zone of inhibition for plant extract against tested bacteria were measured in millimeters.

## 3. RESULTS

### 3.1. Phytochemical Analysis Result:

The phytochemical analysis of ethanol extract of Capsicum fruits showed the presence of alkaloids, flavonoids, free amino groups, phenols, saponins and tannins as shown in Table 1.

### 3.2.The Scolicidal Assay Result:

The percentage of live protoscolices in the sample was calculated by dividing the number of live protoscolices in the sample with the total number of calculated 100× headings. The process was repeated three times and the survival rate was determined. The viability of the control group of protoscolices was 100% as shown in Table 2.

The results revealed that there were significant differences between the ethanol extract of Capsicum groups and the control group at all concentrations and exposure times ( $p < 0.05$ ).

The highest reduction in protoscolices was at 500 mg/ml with a 23%, 9% and 0% survival rate after 5, 10 and 15 mins of exposure time respectively, with significant differences between the means for survival rates of used concentrations ( $p < 0.05$ ). After 15 mins of exposure time there was high scolicidal activity at all concentrations 50, 100, 500 mg/ml with a 22.4%, 19.8% and 0% survival rate respectively with significant differences when compared 5 and 10 mins of exposure time ( $p < 0.05$ ).

The percentage survival rate of the protoscolices decreased with increasing concentrations and exposure times Figure 1.

Table 1: Phytochemical compounds present in the ethanol extracts of Capsicum

Plant	Compounds						
	Alkaloids	Flavonoids	Free amino group	Glycosides	Phenols	Saponins	Tannins
<i>Capsicum sp.</i>	+	+	+	-	+	+	+

Table 2: Protoscolicidal effects of the Capsicum ethanol extract at different concentrations according to the time of exposure

Concentrations mg/ml	% Survival rate after			Mean of concentration	Sig.
	5 min ± SD	10 min ± SD	15 min ± SD		
<b>50</b>	69.60 ± 7.54	56.30 ± 3.67	22.40 ± 3.51	<b>100 ± 0.00</b>	<b>a</b>
<b>100</b>	35.60 ± 3.80	34.40 ± 4.81	19.80 ± 1.65	<b>49.34 ± 21.56</b>	<b>b</b>
<b>500</b>	23.00 ± 2.61	9.00 ± 1.53	0.00 ± 0.00	<b>29.95 ± 8.27</b>	<b>c</b>
<b>Control</b>	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	<b>10.67 ± 10.16</b>	<b>d</b>
<b>Mean of survival rate</b>	<b>57.05 ± 31.63</b>	<b>49.92 ± 34.99</b>	<b>35.55 ± 39.93</b>		
<b>Sig.</b>	<b>a</b>	<b>b</b>	<b>c</b>		

a, b, c, d The values are statistically significant at  $P < 0.05$

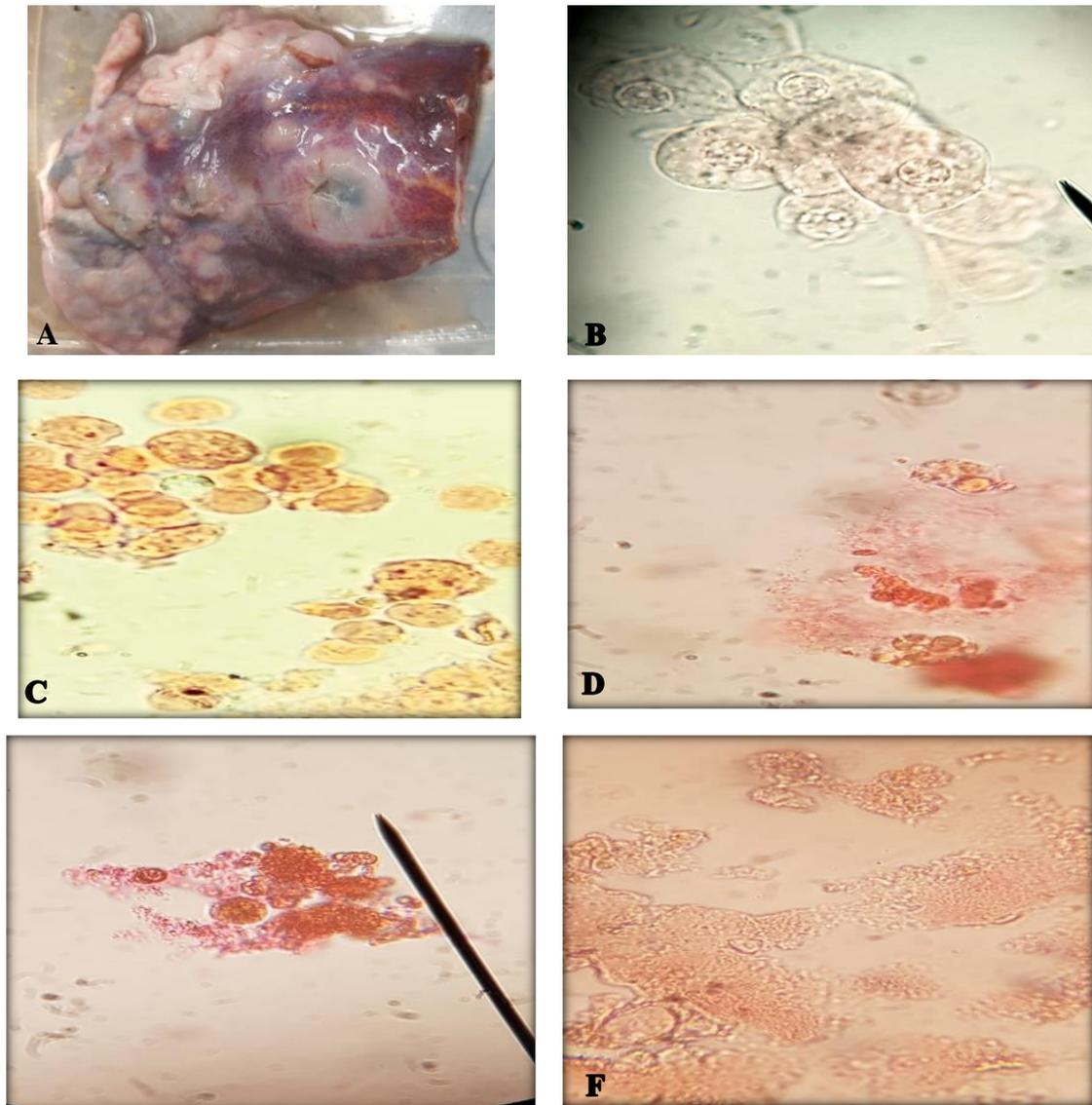


Figure 1: A: hydatid cyst from sheep liver. B: protoscolices of hydatid cyst collected from naturally infected livers of sheep. C: live protoscolices after staining with 0.1% of eosin. D: dead protoscolices treated with 50 mg/ml of ethanol extract of Capsicum Capsicum and stained with 0.1% of eosin. E: treatment with 100 mg/ml of ethanol extract of Capsicum and stained with 0.1% of eosin. F: treatment with 500 mg/ml of ethanol extract of Capsicum and stained with 0.1% of eosin.

### 3.3. Antibacterial Activity Assay and MIC Result:

The current study showed that the ethanol extract of Capsicum had different inhibitory activities against tested pathogens. Whereas it exhibited the highest inhibitory activity against Gram positive bacteria *Staphylococcus aureus* with an MIC value of 125 mg/ml and an inhibition zone of 17 mm, the results did not observe any effect of the plant extract on Gram negative bacteria *Pseudomonas aeruginosa* as shown in Table 3 , Figure 2.

Table 3: Zones of inhibition of and *Pseudomonas aeruginosa*.

**E** ethanol extract of Capsicum against *Staphylococcus aureus*

Bacterial species	Zone of inhibition in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Staphylococcus aureus</i>	17	17	0
<i>Pseudomonas aeruginosa</i>	0	0	0



Figure 2: Zone of inhibition of ethanol extract of Capsicum against *Staphylococcus aureus* with concentrations: 1=250 mg/ml, 2=125mg/ml, 3=62.5 mg/ml.

#### 4. DISCUSSION

in spite of the risk of spillage of hydatid fluid and formation of secondary cysts, surgery remains the best method for removing a hydatid cyst. During surgery, protoscolicidal agents are injected into the cyst to prevent its reformation.<sup>3,9</sup> The WHO has referred to the urgent need for new protoscolicidal agents with fewer side effects and more efficacy.<sup>3,8</sup> Various studies have referred to the protoscolicidal effects of some plant extracts, for example *Allium sativum*, *Nigella sativa*, *Punica granatum* and *Salvadora persica*.<sup>28-30</sup> However, there is still a need for more effective and available agents requiring only a short exposure time. In another direction, hydatid cysts can be infected by bacteria and fungi, such as *Escherichia coli*, *klebsiella*, Streptococci, Staphylococci, *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.<sup>7</sup> Therefore, this study has attempted to find an agent which combines antibacterial and anti-protoscolices activity.

Both traditional medicine and academic researchers have discussed the importance of Capsicum fruits to treat many disorders, syndrome and pathogenic diseases.<sup>12</sup> The bioactive compounds of the plants which include alkaloids, flavonoids, glycoside, phenols, saponins and tannins have been documented for their antimicrobial and antiparasitic activity. The ethanol extract of Capsicum fruits was found to possess most of these compounds. This result is in line with Tasdemir *et al.* and Koffi-Nevry *et al.*<sup>15,31</sup>

The activity of Capsicum extract against protoscolices has not been previously reported. However, the methanol extract of *C. frutescens* leaves has demonstrated significant antihelminthic activity in different concentrations by causing paralysis then mortality of worms.<sup>18</sup> Aqueous extracts of *C. frutescens* also demonstrated 70% death of *Ichthyophthirius multifiliis* during *in vitro* experiments.<sup>20</sup> Our results indicate that

the Capsicum extract worked as a good scolicedal agent in a short exposure period. It was shown to have a destructive effect on *E.granulosus*. This activity, considering its low cost and high safety make it a good antihelmintic substance that can be used during surgery to prevent the recurrence of hydatid disease. Generally, different concentrations were proved to have significant scolicedal effects at  $p \leq 0.05$  in the present work.

It has been observed that 70% ethanol extract of Capsicum showed the best antifungal activity compared with water and acetate extracts. Moreover, Capsicum extracts displayed high diffusion, when using the well method to evaluate the antimicrobial activity, due to direct contact between the extract and the agar.<sup>16</sup> Therefore, ethanol extract was chosen in this study to evaluate its activity using the well method.

In this study, ethanol extract obtained from hot Capsicum exhibited antibacterial properties against Gram positive pathogenic bacteria *S. aureus*, with no activity against Gram-negative bacteria *Pseudomonas aeruginosa*. These results disagreed with previous studies,<sup>32-33</sup> which showed that Capsicum extracts had antibacterial properties against both Gram-positive and Gram-negative bacteria. While our findings agreed with those of Koffi-Nevry *et al.*,<sup>15</sup> which showed activity of Capsicum extract against some bacterial species (*Staphylococcus aureus*, *Salmonella typhimurium*, and *Vibrio cholerae*) and no efficacy against *Shigella dysenteriae* and *Pseudomonas aeruginosa* and *Escherichia coli*, this difference could be attributed to the low permeability of Gram-negative bacteria membrane. This membrane consist of uneven and inflexible bilayer of lipopolysaccharides (LPS) and phospholipids. This led to specific uptake channels and nonspecific porins are implanted, which caused reducing passive diffusion of hydrophobic compounds to selective size of hydrophilic solute.<sup>34</sup>

In spite of the fact that the mechanism of action for the extract was not studied, the activity of phytochemical compounds can be belong to blockage in the cell wall synthesis by formatting complexes between protein and tannin. Falvonoids can be work as same as tannin by inhibiting cell wall of bacteria through formatting complex with different types of proteins. The saponins are responsible on outflow of important constituents from the cell. Terpenoids can cause dissolution of the bacteria cell wall.<sup>35</sup> Phytochemical analysis of Capsicum ethanol extract detected these compounds (flavonoids, saponins and tannins). Many studies have revealed that Capsaicin alkaloids are the most active compound in the fruit extract of Capsicum. It has been demonstrated to have different biological thermogenic influence effects, anti-inflammatory, antilithogenic and beneficial effects on the gastrointestinal system.<sup>36</sup> It has been suggested that capsaicin and dihydro-capsaicin prevent cariogenic processes, and decrease the level of acid secretion and biofilm formation by *Streptococcus mutans*.<sup>37</sup> Yeast DNA micro-array methods were used to understand the antimicrobial mechanism of capsaicin. It was found that capsaicin induced 39 genes from about 6,000 genes; these genes are responsible for membrane biosynthesis genes, multi-drug resistance transporter genes and genes encoding stress proteins.<sup>38</sup>

## 5. CONCLUSION:

The Capsicum extract is an ideal scolicedal agent which is defined by its potency at lower concentrations, high efficacy after only short exposure times, high availability and ability to be prepared rapidly. No studies have previously used it as a scolicedal agent. This is the first report on the scolicedal activity of Capsicum extract, in conjunction with its antibacterial activity. Nevertheless, there is a need to explore *in vivo* scolicedal activity of the ethanol extract of Capsicum.

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