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Anthelmentic Effect of Ethanol Leaves and Hexane Seed Extarction of Capparis Spinosa on Earth worm Motility and Echinococcus Granulose Protoscolices, in Kurdistan Region, Iraq

Lokman T. Omer Al-Barwary

University of Duhok, Faculty of Veterinary Medicine

Abstract: ***The present study was conducted to assessment in-vitro anthelmintic activity of ethanolic and hexane extract from leaves and seed of plant Capparis spinosa. The anthelmintic activity of plant leaves and seed is investigated through using earthworm (Lumbricus terrestris) and on viability of protoscolices Echinococcus granulosus. Various concentrations of ethanol (leaves) and hexane (seeds) extract (200 and 300 mg/ml) from both extract of capparis spinosa were tested, for determination of death and paralysis time , and 5, 10,20 mg/ml for both extract for assessment mortality percentage protoscolices Echinococcus granulosus and fenbendazole was included as reference standard.The mortality of protoscolies with both extract after 2hr reach 90-100%, however, the results related that the leave extract was more effective on the motility of earth worms, since the paralysis occurs after 8.5 minutes and death 21 minutes during using 200mg/ml. and the time need to paralysis and death occurs by using 300mg/ml after 7.3minutes and 18 minutes respectively. The results of study indicate, that plant Capparis spinosa, possesses strong anthelmintic activity***

Keywords: Capparis spinosa., Lumbricus terrestris (earth worms), protoscolices Echinococcus granulosus.

1. Introduction

Capparis spinosa (belonging to the family Capparidaceae) is a xerophytic plant growing in a broad range of weather conditions, varying from dry deserts to cooler altitudes of mountains (Puganair 1989).

This plant bear rounded fleshy leaves and large white to pinkish flowers (Ramezani et al 2008). Habitat and distribution of capparis spinosa common in the mountains, moist and dry steppic plains, on limestone, cliffs, on gravely soil, rocky places, in roadside cuttings, in mountain valleys Common in the lower forest zone up to 900m altitude and this plant found all parts of Kurdistan (shahbaz 2013). This plant needs a semiarid climate. Mean annual temperatures in areas under cultivation are over 14 C and rainfall varies from 200 mm/ year (Mohammad et al., 2012). Many species of capparis are reported from Iraq, from northern to southern of the country (Chakararty 1976). Traditionally diarrhea and hemorrhoids have been treated with Capparis spinosa roots and fruits. Also farmer in the 10th century use aqueous extract from it is roots for disinfecting. Worldwide, medicinal plants, mineral, and materials from animal's sources were used as traditional remedies by human due to healing prosperities. As well as human for build and maintain health, use medicinal plant for stave off disease, or promote recovery from disease or infection (sher H. and Alyemeni M.N, 2010). In Iran, and in different other country Capparis spinosa is one medicinal plant used to have highly diverse of medicines (Azaizeh et al., 2003). Eighty percent of the population in Africa, according to WHO use traditional medicine for healthcare, and in many western countries natural treatment very common (Larsen and Smith, 2004 ; Al-Quran,2008).

Lumbricus terrestris earth worms occur in the highest numbers in grassland, eat a variety of organic matter, including dead leaves and of the plant debris, soil micro-organisms. The earth worms are hermaphroditic and move along by waves of muscular contraction, and have been scientifically classified under Phylum Annelida, class Oligochaeta, Order Haplotaxida, Suborder Lumbricidae and family (McLaughlin 1986). The antihelminthic activity of C. spinosa extract may be due to the presence of tannin, which is a polyphenolic compound capable of producing the same effect as that of some synthetic phenolic antihelminthics, such as niclosamide, oxyclozanide and bithionol. These compounds are known to interfere with energy generation in helminth parasites by uncoupling parasite specific fumarate reductase mediated oxidative phosphorylation (Bate-Smith 1962). Also antihelminthic effect of tannins is that they can bind to free proteins of host GIT(Athasidou 2001) or glycoprotein on the parasite cuticle(Thompson and Geay 1995) and ultimately causing parasite death.

Hydatid disease was beheld as one of the big health problem in Iraq and all over the world (Andersen et al., 1997 and Al-Hammo, 1999). This problem caused by the larval stage (metacestodes) of Echinococcus granulosus. Adult stage inhabits the GIT of carnivores, human get the infection by ingestion of ova that fired to the environment by the final host (Zeibig, 1997). Now are three traditional methods for treatment of hydatid disease: 1-Surgery. 2-Puncture and reaspiration (PAIR). 3-Chemotherapy. The third one is still considered as the last resort for the treatment, and is only indicated to prevent complication and for untreatable cases (WHO, 1996). Newly, many workers look forward for the use of natural products in the treatment of many diseases, including echinococcosis. Al-Tae (1996) and Ali (1999).Al-Hammo(2002) showed that Matricaria chamomilla Linn. and Cyperus rotundus Linn. Posses restrained effect on the viability of hydatid cyst protoscolices in vitro. Mahmoud (2002) showed that the alkaloidal extract of peganum harmala seeds caused reduction in the viability of

protoscolices of human and sheep origin. The Kurdistan weather and geographically location provided an ideal media for the growth and nourishment of different plant species even medicinal plant including Capparis spinosa, but until now have not been used as medicinal plant. There for we aimed to evaluated the Ethanol and hexane extract of the plant Capparis spinosa on the earthworms as well as *Echinococcus granulosus protoscolices*

2. Material and Methods

2.1 Plant Material

The leaves and seeds of Capparis spinosa were collected in August 2013 in Duhok districts specially Mallta area in Kurdistan region. the collected plants were authentified by Prof. Saleem Esmael Shahabz ,at faculty of Agriculture, University of Duhok , Kurdistan region. The collected seeds and leaves of the plant were washed and dried in by electric blender then crushed to form coarse powder and subjected to soxhelt extraction by using 80% ethanol and hexane. the extracts were concentrated by rotary evaporator before testing on the used parasites.

2.2- Chemical Analysis as Show in the Figure (1)

For chemical analysis, the prepared extracts were sent to Eastern Mediterranean University, Gazimaguse North Cyprus / Faculty of Pharmacy, the analysis were done by FT-IR spectrometer (65-Perki Elme) .

2.3 Experimental Parasites

2.3.1 The first experimental were carried out in adult earthworms (Lumbricus terrestris) due to anatomical resemblance with the intestinal roundworm parasites. They were collected from moist soil and washed with water to remove all matters.
Ethanolic (leaves) or hexane (seed) extracts (200 mg/ml or 300 mg/ml) were prepared (Mustafa 2012). Six worms were placed in each formula and the time for paralysis was recorded when no movement of any sort was observed. Time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water

2.3.2 The second experiment was carried out on Echinococcus granulose protoscolex. Protoscolices collection and suspension:

In this study, hepatic hydatid cysts of infected sheep were obtained from the municipal abattoir in Duhok city, Iraq. Protoscolices were removed from the cysts by aseptic techniques, washed in several changes of sterile phosphate buffer saline (pH 7.2). Protoscolices were then suspended in a sterile hydatid fluid that contains 2% organic solvent, Dimethyl sulfoxide (Farjou and Al-Hussainawi, 1984).The samples then put in test tubes, 2ml\tube, 500 Protoscolices\ml of the suspension.

All used hydatid cysts possessed a mean viability of at least 90%, which was determined by peristaltic movements of protoscolices, negative staining with 0.1% aqueous eosin and flame cell movement (Smyth and Barrett, 1980). The collected protoscolices were treated with different concentrations of the ethanolic (leaves) and hexane (seed) extract (20, 10, 5 mg /ml). The first group was incubated at 4°C, and the second group was incubated at 37°C. For each group, the viability of potoscolices was examined at different time of exposure (10, 20, 30 minutes). The viability was tested microscopically by adding 10 µl of eosin solution to 10 µl of protoscolices for 15 min. (0.1 mg of eosin stain was dissolved in 100 ml distilled water). Stained protoscolices were considered as dead while unstained protoscolices were recorded as a live.

3. Results and Discussion

Table 1: show the effect of both extracts on mortality % of Protoscolices

concentration	Extract	30 min	1hr	2hr
50mg/ml	Ethanol	36.3	52	93
	Hexan	27.2	49	89
100mg/ml	Ethanol	40.6	89.5	100
	Hexan	39.8	88	100
200mg/ml	Ethanol	74.7	100	100
	Hexane	69.3	93	100
10mg/ml	Fenbendazol	95	100	100
Control 4C		2.5	3.1	4.8

Table 2: Show anthelimenthice activity of both extracts (ethanol and hexane of Capparis spinosa leaves and seeds) on movement of earth worms

Extract	Concentration Mg/ml	Paralysis time (minutes)	Death time (minutes)
Ethanol extract	250	28.2	40.1
	300	19.3	38.5
Hexan extract	250	8.5	21
	300	7.3	18

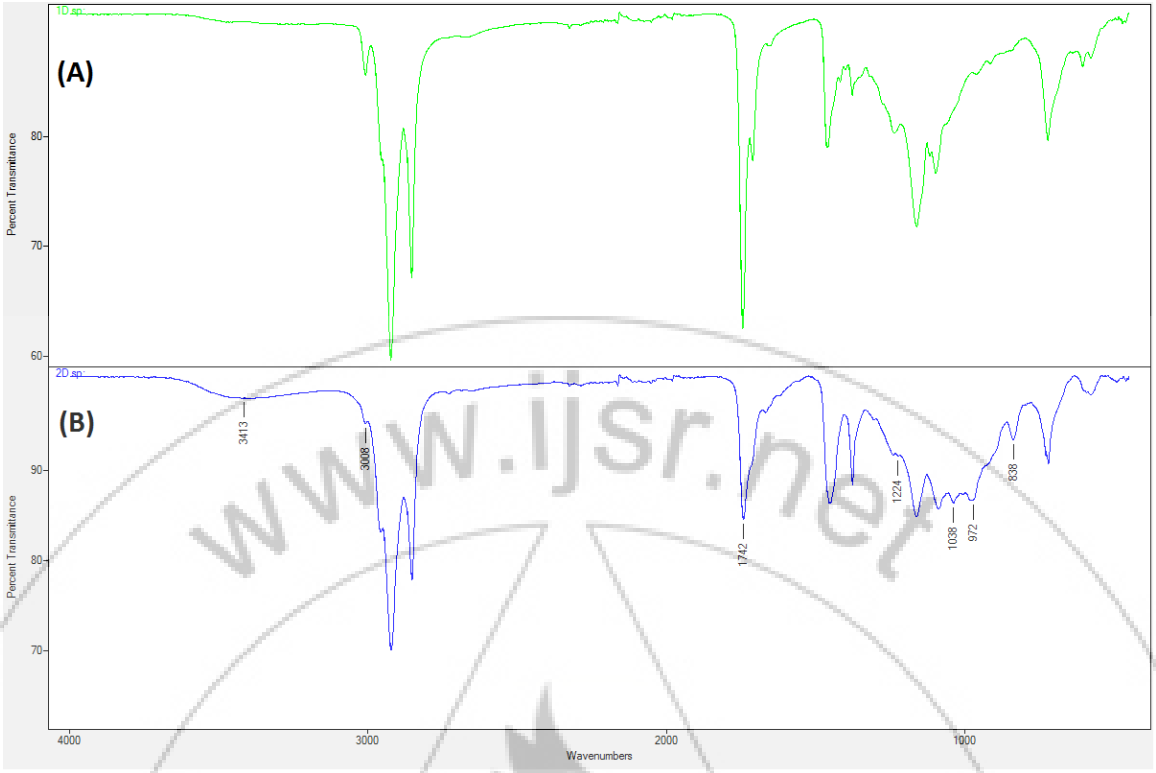

Figure 1: Show the chemical analysis of both extract

Fourier transforms infrared spectroscopy (FT-IR) Analysis

A- Hexane extraction

B- Ethanol extraction

These differences reveal two chemical compositions,

In case Hexane extraction seed (a) it has strong peak observed less than 3000 cm-1 at 2900 cm-1 due to C-H stretching and the bands at 1470 cm-1 and 1742 cm-1 are due to C-H bending and C=O stretching respectively. (a) Shows a band at 2900 cm-1 strong, and at (1470 cm-1) C-H bending which refers to the alkane group.

For (b) Ethanol extraction of *Capparis spinosa* leaves FT-IR spectroscopy peaks of O-H stretching (3413 cm-1), C-H stretch (2920 cm-1), C-O stretch (1038 cm-1), and C=O cm-1 (1742 cm-1) are present. O-H group here refers to the alcohol.

4. Discussion

Capparis spinosa is classified as medicinal plant that contains numerous biologically active compounds (Sharaf et al., 2000). As we found in our results the effect of two extraction of Capparis spinosa by ethanol and hexane has anthelmentic effect. In table 1 show both extractions after exposure the protoscolexe of Echinococcus granulose the mortality reach between 100 and 93 % respectively and after 2hr. the mortality about 100% while the the control show after 1hr. and 2 hr. 3.1 and 4.8% respectively.

Whoever, AL-Abody and AL-Aqais 2013 found after 1hr reach mortality 100% and the control reach 9.7% when they use leaves Aqueous extracts of capparis spinosa.

And the table 2 show the effect of two extract on the activity of earthworm in this table we found there is difference between two extract the hexane extract with concentration 200mg/ml led to paralysis after 8.5min and death occur after 21min on the other hand the effect of ethanol extract had effect of paralysis after 28.2min and death after 40.1 min with 200mg/ml

And with concentration of 300mg/ml the paralysis of earthworms with hexane and ethanol extract 7.3min and 19.3min respectively and death occur with 300mg/ml of hexane extract after 18min, and 38.5min time need to death occur with the same concentration of ethanol extract.

While Mustafa 2012, found the paralysis occure after 10.96min and 6.16min with concentration 200mg/ml and 400mg/ml respectively when she use ethanolic extract.

5. Conclusions

These pharmacological studies on plant Capparis spinosa for anthelmentic activity reveal that the ethanolic and hexane extract show the anthemintic activity with there is no statistical difference between two extract. Even there are chemical differences between them

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