

## **Anticercarial Activity of The Plant *Peganum harmala* (Harmal) Crude Extracts**

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### **Abstract**

In the present work seeds of *Peganum harmala* were extracted by distilled water and methanol. The extracts were then tested as anti-cercaria in vitro which were collected from the natural infected snails with *Schistosoma mansoni* cercaria. All concentrations of 250 ppm up to 1000 ppm showed 100% mortality rate during the first half an hour of exposure for all used extracts. Serial dilution concentrations of both water and methanolic extracts (125/67/35 and 15 ppm) were tested and found to exhibit different mortality rates with an LC<sub>50</sub> 291.27, 111.05, 64.74 and 47.95 ppm for the water extract and 97.92, 53.49 & 26.95 methanolic extract successfully for the diluted concentrations of (125, 62, 31 & 15 ppm). On the other hand the methanolic extract showed a 100% mortality rates after 3 hours of exposure for the whole concentrations.

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These results highlight that the methanolic extract was more active for treatment of such cercariae. However, the present study, recommend more explation and investigation for active ingredients for the use of the plant in the field and contribution in schistosomiasis eradication or treatment.

### **Introduction**

Schistosomiasis is a parasitic disease and endemic disease. It affects millions of people in Africa and South America (Bilia *et al.*, 2000). In Brazil, it affects over 8 million people and about 30 million live in hazard areas due to the presence of infected snails.

Schistosomiasis may damage visceral organs, especially the liver, posing risk to life. There are different kinds of schistosomiasis, but in all cases, the reproductive cycle involves aquatic snails. The parasite multiplies into hundreds of cercariae that can penetrate the intact human skin of those who are exposed to infected waters after leaving the snails (Hostettman, 1993). Chemotherapy is a general strategy for schistosomiasis control; but another more interesting is one that interrupts the disease vital cycle by snail's or cercariae's elimination.

The use of plants with molluscicidal properties is simple, inexpensive and appropriate for the local control of the snail vector (Hostettman, 1993). Since the discovery of highly potent

saponins in *Phytolaccadodecandra* (Phytolaccaceae) berries (Marston & Hostettman, 1993), naturally occurring molluscicides have received considerable attention and the number of reports on the use of plant-derived molluscicides has increased considerably (Hostettman, 1993; Oliveira *et al.*, 2006; Sousa *et al.*, 2008).

In the plant kingdom there are thousands of plant known and unknown that yield medicine or drug of great use to man one of them was used as molluscicide and anticercariae for eradication and control of the disease. However, the potential use of plants for snail control has recently received considerable attention (Perrett and Whitfield, 1996). However, it is necessary in the control of the disease improving the sanitary condition, prevent water contact and use biological controller fish (Sturrock, 1996). Moreover there are many findings which prompt research on eradication and control of such disease. *Peganum harmala* belong to the family zygophyllaceae vernacularly named as Harmal traditionally its seeds have been used for the treatment of helminthes infection. Chemically the plant is characterized by the presence of harmaline and harmol as principle alkaloids .In addition to harmine, pyganine and gregarine are also present. The alkaloids extracted from the plant seeds have proved to possess antitumoral activity .The plant had also shown strong activity against haemosporidian protozoan parasites, *Theileria Sergenti* and *Babesia gigemina*

and a potent anthelmintic activity against *echinocooccus granulosus* (Sousa *et al.*, 2008).

The objective of this study is to investigate the cercaricidal activity of the medicinal plant *Peganum harmala* by using series concentrations of the both water and alcoholic extracts of the seed against the cercariae of *S. mansoni* collected naturally from infected snails.

## **Material and Methods**

### **The plant samples**

Seeds of *Peganum harmala* was purchased from a local market (about 500 gm), authenticated and identified in the Herbarium of Medicinal and Aromatic Plants Research Institute. There were grinded coarsely and the powder used immediately for extraction by water and methanol.

### **Preparation of alcoholic extract**

100 gm from the powder of the plant was weighted by using sensitive balance and put in the conical flask of 1000 ml size. 500 ml of methanolic alcohol was added to the powder. The mixture was then put on the shaker for about 4 hours in room temperature. The mixture then filtered by using cotton in a large liter size at 2 beakers and allowed to dry by using water bath of 60 °C occasionally for 24 hours. The extracted powdered was then crushed by help of spatula and deposited in

small bottle of 50 ml size and then stored at 4 °C for further use.

At the time of the biological test, 1mg (1000 µg) was added to 1ml of distilled water to give a concentration of 1000 ppm, followed by a serial dilution by adding equivalent amount of distilled water 1:2 also to give the following concentrations 500, 250, 125, 62.5, 3.25, 15,625 ppm respectively.

**Preparation of water extract:**

100 gm from the powdered seeds of *Peganum harmala* was weight by using sensitive balance and put in the conical flask of 1000 ml size. 400 ml of boiled distilled water was added to the powder. The mixture was then put on to the shaker for about 4 hours in room temperature. The mixture then filtered by using cotton in a large beaker of 2 liters size. Then the stalk solution was moved from the beaker to medium size bottle (200 ml) for storage at 4 °C until the time of usage.

The concentration of stalk solution was calculated by taking 2ml before the storage and deposited and a Petri dish of known weight, then allow to dry overnight. After the dryness the beaker was weighted again and the difference gave the concentration of the dry plant within 2 ml of stalk solution. (Then divided by 2 to give the concentration of stalk solution in 1 ml.)

### **Biological Assay**

Snails of *Biomphalaria* sp. naturally infected with miracidia of *S. mansoni* eggs was collected from a narrow canal in Alkriab village (15 km East South of Khartoum North) by using a deep scooping method which was described by Malek, (1962).

The snails were put in large dishes and closed by wet cotton, snails were collected to the Parasitology laboratory of Medicinal and Aromatic Plants Institute, there they were put individually in small beakers (50 ml) and subjected to sudden shock of light and allowed for the shedding of cercariae which are observed by naked eye and magnified lens after 1 hour from the exposure. The cercariae were taken under inverted microscope for identification and differentiation from other trematodes.

For the evaluation of plant bioavailability 24 well microtitre ELISA plates were used Each column was used for only one concentration of plant extract i.e. 4 replicates (each plate was used for 6 different concentrations for both water and ethanolic extracts).

Ten cercariae were taken by using plastic Pasteur pipette under the inverted microscope and deposited in each well.

The motility of the cercariae was observed after each hour by the morphological and physiological changes of the organism and the average mortality was tabulated.

## Statistical Analysis

Data obtained in the present study was analyzed for detecting the lethal concentrations using ManiTab analytic program - Reliability/Survival- Probit analysis (Version 13).

## Results

All the concentrations used in this experiment (250 ppm upwards to 1000 ppm) had shown 100% mortality at the first hour after the exposure of *S. mansoni* cercariae for both water and alcoholic extracts of *P. harmala*.

Table (1) and Fig (2) show the mortality % (percentage) of cercariae of *S. mansoni* for the plant water extract of the concentrations 125, 62, 31 and 15 ppm .The highest mortality was 100% and the extract showed a lethal concentration(50%) of 291.27, 111.05 , 64.74 & 47.9 for the serially diluted concentrations ( 125, 62, 31,& 15 ) respectively.

**Table (1). The mortality % of the plant water extract against cercariae of *S. mansoni* .**

Time	Conc *(125 ppm)	Conc *(62ppm)	Conc. * (31ppm)	Conc. * 15 ppm	Cotrol	LC50
½Hour	57	26	9	5	0	291.27
1hour	87.5	47.5	17.5	9	30	111.05
2hours	92.5	52	26	18	42.5	64.74
3hours	100	60	32	22	45	47.95

\*concentration

One way ANOVA test (p =0.000)

Table (2) and Figure (3) show the mortality % of the plant ethanolic extract for the concentrations 125, 62, 31 and 15 ppm against cercariae of *S. mansoni* for three subsequent hours of observation time. The highest mortality was 100% reported for all concentrations after 3 hour. However, the methanolic extract showed a lethal activity of 50% mortality (LC<sub>50</sub>) 97.92, 53.49 & 26.95 for the serially diluted concentrations of the extract (125, 62, 31 &15) respectively after ½ hour, 1 hour and 2 hours, whereas after 3 hours the extract gave 100% mortality for the whole diluted concentrations.

**Table (2). Mortality % of the plant methanolic extract against cercariae of *S. mansoni* .**

Time	Conc. *(125pp)	Conc. *(62ppm)	Conc. *(31ppm)	Conc. *(15ppm)	Cotrol	LC50
½hour	58	40	31	15.5	0	97.92
1hour	77.5	55	42	12	30	53.49
2 hours	97.5	77.5	50	20	42.5	26.95
3 hours	100	100	100	100	45	-

\* Concentration

**One way ANOVA test ( p =0.456)**

## **Discussion**

In the Sudan, there are many local plants screened and tested in the laboratory and proved to have molluscicidal activity (El-kheir and El-Tohami, 1979; Ayoub, 1985; El-Sheikh, 1994) and ( Elgazali, et al.,1998)

In the present study this plants had revealed a very efficient

anti-schistosomal activity against water stages, hence this activity hinder the life cycle of the parasite and play an important role in biological control program. The methanolic extract was more efficient in comparison to the water extract because the lower concentration of 15 ppm showed 100% mortality after 3 hours from the time of exposure. Moreover, the plant in many studies showed antiparasitic and anticancer activity (Misra P. *et al.*, 2008, Lala S. *et al.*, 2004, Derakhshanfar A, Mirzaei M, 2008 and Li Y, Liang F, Jiang W, *et al.* 2007).

Elshiekh, 1994 studied the plant *Balanites aegyptiaca* water and alcoholic extract also highlight similar result to this study. However it is reported that both of seeds of *Peganum harmala* and leaves of *Solanum nigrum* had chemical effect on cercaria (Osman, 2000). Moreover both plants showed high mortality to snails too (Osman, 2000). In this study the plant extracts used in this study were characterized generally by their less toxicity and high threats hold of safety, hence it is highly recommended as a good method to control disease for both of the cercariae and snails cercaria and snails.

### **Recommendation**

According to the above result shown that we can recommend the use of *Peganum harmala* for treatment of *Schistosoma mansoni* targeting to several aspects of life cycle through it can attack the vector and water stages and had proved to be

valuable against both water stages and intermediate host..Moreover, plant can be used either as powder form for spraying the canals especially in its methanolic extract rather than aqueous extract. Also it is possible for people who are attaching by water resources to use oil for skin lotion against cercariae attack.

Further study should be carried against the adult stages of *S. mansoni*, and other type of *Schistosoma*, as well as identification of the active ingredients to establish the baseline for pharmaceutical formulation. However, the plant has a broad-spectrum activity against different types of helminthes.

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