

ASSAYING INSECTICIDAL AND WORMICIDAL ACTIVITIES IN CRUDE EXTRACTS OF LEAVES AND SEEDS OF *PORTULACA OLERACEA* L.

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ABSTRACT

Methanolic extracts of leaves & seeds of *Portulaca oleracea* were assayed for insecticidal and wormicidal activities in different concentrations (1mg to 100 mg/mL) on *Tribolium castaneum*, *Sitophilus oryzae* and *Lumbricus terrestris* along with positive, negative control and standard drugs. The crude extract showed no mortality in all doses in insecticidal activity but significant dose dependent mortality was observed in wormicidal activity test.

Key-words: insecticidal activity, wormicidal activity, *Portulaca oleracea*, human diseases, herbal medicine.

INTRODUCTION

Pest is a problem for storage of cultivated crops and medicinal plants in high temperature regions (Sindh, part of Balochistan and the Punjab) of Pakistan where as worms are problem in human body especially in children causing diarrhea, dysentery and skin diseases (Nadkarni, 1982; Chopra *et al.* 1986). This plant, *Portulaca oleracea* (Portulacaceae), grows abundantly throughout Pakistan (Ali & Nasir, 2001) and local people consume it as vegetable especially as salad (Nadkarni, 1982; Chopra *et al.*, 1986). A number of compounds like ω -3-fatty acid glycosides, proteins, vitamin C, K-salt, urea, oleic acid, alkaloids, flavanoids and saponins are reported from *P. oleracea* (Kirshnamurthi, 1972; Nadkarni, 1982; Watt and Brandwijk, 1962; Omara-Alwala and Thomas., 1991). *P. oleracea* has many pharmacological applications e.g. in blood pressure, clotting time, anti-inflammation, cholesterol (LDL) and coronary spasm (Bharadwaj, 1988). In the present study insecticidal and wormicidal activities of methanolic extracts of *P. oleracea* were determined.

MATERIALS AND METHODS

Preparation of Crude Extract:

Whole fresh plant material of *P. oleracea* was purchased from local market, Karachi, Pakistan. The sample material was deposited in the herbarium of the Department of Pharmacognosy with code number PO-01SS-1502/2007. The fresh plant material (5kg leaves) was first cleaned by removing the debris and then chopped into small pieces. This material was soaked in methanol (6 liters) for percolation at least 15 days at room temperature. After percolation, the methanol extract was obtained by filtration and the residue was once again extracted with methanol. This procedure was repeated three times and all three extracts combined together and then evaporated on rotary evaporator under reduced pressure. After the evaporation of methanol, a thick dark colored residue was obtained. Later it was dried on a rotary evaporator and the dried powder (480g) was collected. A part of this crude extract of leaves was used for further studies. Same procedure was used for the extraction of 1kg seeds.

INSECTICIDAL ACTIVITY TEST

This test was carried out on insects, *Tribolium castaneum* and *Sitophilus oryzae*. During experiments organic solvents methanol, ethanol, acetone (Merck, Germany) and standard drug, Permethrin, (Coopex) from Reckitt & Benckiser, Pakistan were used. The stored grain pests were reared in laboratory under control conditions (temperature & humidity) in plastic bottles containing sterile breeding media. The insects of uniform age and size (10 for each concentration) were used for experiments. Crude methanolic extract (200 mg) was dissolved in 3ml methanol and uniform solution was prepared. Rest of the procedure as described by Collins (1998), Tabassum

(1997) and Abbott (1925) was adopted for the experiment. The results and readings were taken at least three days. Percentage for inhibition or percentage mortality, the following formula was used.

$$\text{Mortality} = 100 - \frac{\text{Number. of insects alive in test}}{\text{Number of insects alive in control}} \times 100$$

In positive control standard insecticide (Permethrin (Coopex) from Reckitt & Benckiser, Pakistan) was used. During experiment effective concentration ($235.9 \mu\text{g}/\text{cm}^2$) was used against all test insects. In negative control volatile solvent was used during experiments.

WORMICIDAL ACTIVITY TEST

Earthworm, *Lumbricus terrestris*, was used as the test species in this experiment. Before starting the experiment the earthworms, bred in the laboratory, were washed with distilled water to remove the mud and other material attached to them. Later four earthworms placed in each Petri-dish containing different concentration of methanolic extract (1, 5, 10, 25, 50, 75 and 100 %). The earthworms were observed for their spontaneous motility or paralysis and evoked responses to pin prick which were scored for 0 to 4. The paralytic score was recorded at different time intervals. Immediately after inhibition of response to pin prick, the worm were place in fresh water and observed for recovery. Duration required for the final recovery or death was recorded. Mean paralytic score was plotted against time and compared with Piperazine (3% solution) as reference standard (Kumar and Shivkar, 2003).

Table 1. Insecticidal activity of *P. oleracea* (methanolic extract of leaves)

Dose	<i>Tribolium castaneum</i>		
	No. of survivor	Time of onset of drug action (immobility time)	% Mortality
1 mg	10	10min (2 P, R)	0
5mg	10	10 min (4P, R)	0
10mg	10	-	0
25mg	10	-	0
50mg	10	-	0
75mg	10	-	0
100mg	10	10min(2P, R)	0

Table 2. Insecticidal activity of *P. oleracea* (methanolic extract of seeds)

Dose	<i>Tribolium castaneum</i>		
	No. of survivor	Time of onset of drug action (immobility time)	% Mortality
1 mg	10	-	0
5mg	10	-	0
10mg	10	-	0
25mg	10	10min(2 P, R)	0
50mg	10	10min (3 P, R)	0
75mg	10	10min (3 P, R)	0
100mg	10	10min (3P, R)	0

Name of Insects	% Mortality	
	+ ve Control	- ve Control
<i>Tribolium castaneum</i>	100	0

Concentration of Standard Drug = $235.9 \mu\text{g}/\text{cm}^2$
 +ve control = Permethrin (Coopex) Standard drug
 -ve Control = solvent

Name of Insects	% Mortality	
	+ ve Control	- ve Control
<i>Tribolium castaneum</i>	100	0

Concentration of Standard Drug = $235.9 \mu\text{g}/\text{cm}^2$
 +ve control = Permethrin (Coopex) Standard drug
 -ve Control = solvent

RESULTS AND DISCUSSION

The extracts of leaves and seeds of *P. oleracea* in different concentrations were found to be non-toxic (zero mortality in *T. castaneum* and *S. oryzae*) as compared to standard, positive and negative controls. But the insects showed paralysis and recovery (Table 1- 4).

Table 3. Insecticidal activity of *P. oleracea* (methanolic extract of leaves)

Dose	<i>Sitophilus oryzae</i>		
	No. of survivor	Time of onset of drug action (immobility time)	% Mortality
1 mg	10	30min (2 P, R)	0
5mg	10	20 min (4P, R)	0
10mg	10	-	0
25mg	10	-	0
50mg	10	-	0
75mg	10	-	0
100mg	10	10min(2P, R)	0

Concentration of Standard Drug = 235.9 $\mu\text{g}/\text{cm}^2$
+ve Control = Permethrin (Coopex) Standard drug
-ve Control = solvent

Name of Insects	% Mortality	
	+ ve Control	- ve Control
<i>Sitophilus oryzae</i>	100	0

Table 4. Insecticidal activity of *P. oleracea* (methanolic extract of seeds)

Dose	<i>Sitophilus oryzae</i>		
	No. of survivor	Time of onset of drug action (immobility time)	% Mortality
1 mg	10	-	0
5mg	10	-	0
10mg	10	-	0
25mg	10	10min(2 P, R)	0
50mg	10	10min (3 P, R)	0
75mg	10	10min (3 P, R)	0
100mg	10	10min (3P, R)	0

Concentration of Standard Drug = 235.9 $\mu\text{g}/\text{cm}^2$
+ve Control = Permethrin (Coopex) Standard drug
-ve Control = solvent

Name of Insects	% Mortality	
	+ ve Control	- ve Control
<i>Sitophilus oryzae</i>	100	0

Table 5. Assessment of anthelmintic activity of *P. oleracea* (methanolic extract of leaves)

Treatment Doses of <i>P. oleracea</i> (mg)	Spontaneous activity & time								
	With in 1 min	5min	10min	15min	30min	1hr	2hr	4hr	24hr
control	N	N	N	N	N	N	N	N	N
1	0+	2	2	2	2	2	2	2	A
5	0++	2	2	2	2	2	2	2	A
10	0++	2-	2--	2---	3-	3-	3--	4	D
25	0+++	3-	3-	3--	4	4++	D	-	-
50	0+++	1-	2--	3-	3	4++	D	-	-
75	0+++	1-	2--	3--	4+++	D, E	-	-	-
100	0++	3---	4+++	D, E	-	-	-	-	-

(For 15 minutes movement was fast, than slow that was dose dependant reduction in motility and touch evokes response, patch like blisters on body which swell with in 10 min, at 25, 50 and 75% body swells, and at 100mg body contract or shrink)

Results of anthelmintic activity of crude methanolic extracts of leaves and seeds of *P. oleracea* in different concentration are given in tables 5-6. All crude extracts were evaluated at 1, 5, 10, 25, 50, 75 and 100 mg/ml concentration. Results of leaves crude extract showed that 1mg dose increased the activity and slowed down the movement on 5mg dose. The response of worms was immediate on 5, 10 and 25mg dose. 50mg dose resulted in

slow movement of the worms and blisters on the body. In case of 75 and 100mg/mL doses blister appeared immediately on the bodies and worms swelled within 10 minutes.

Table 6. Assessment of anthelmintic activity of *P. oleracea* (methanolic extract of seeds).

Treatment Doses of <i>P. oleracea</i> (mg)	Spontaneous activity & Time								
	With in 1 min	5min	10min	15min	30min	1hr	2hr	4hr	24hr
control	N	N	N	N	N	N	N	N	N
1	0++	2	2	2	2	2	2	2	A
5	0++	2	2	2	2	2	2	2	A
10	0++	2-	2- -	2- - -	3-	3-	3- -	4	D
25	0 +++	3-	3-	3- -	3-	3- - -, E	3- - -, E	D	-
50	0 +++	1-	2- -	3-, E	D, S	-	-	-	-
75	0 +++	1-	2- -, E	3- -	4+++, E	4+++, E	D	-	-
100	0 +++	3- - -	4+++	4, E	4, E	D	-	-	-

Number of replicates = 4 worms; Alive = A; Grade 0 = spontaneous motility; Grade 1 = Moderate motility; Grade 2 = less motility; Grade 3 = reduce touch evoke response; Grade 4 = total paralysis; Grade N = Normal; Grade D = Death; Secretions = S; Edema = E; Size reduction = ↓; Intensity = high+; moderate high ++; very high+++; low - ; moderate low - - ; very low - - -.

Immediately there is contraction and relaxation then movement reduced, patches appeared like blister at body and mouth swell, difficulty in movement.

Seeds extract of *P. oleracea* exhibited quick activity at 1mg dose, the worms started jumping immediately for few seconds then its movement slowed down with the passage of time and blister like patches appeared on the bodies. On 5mg dose worms jumped for 5 seconds and patches appeared on the bodies and mouth swelled. On 10mg dose the worms began jumping for 2 seconds and patches appeared immediately on the bodies and mouth swelled. Twenty five mg dose made increased these activities worm jumped rapidly and very high, contracted highly and felt difficulty in movement, mouth swelled. The higher doses (50, 75 and 100mg per mL) resulted in immediate contraction and swelling of the worms' bodies and death within 30 minutes to 1 hour (Table 5-6).

The results of this study on insects showed that *P. oleracea*, has no insecticide/pesticide activity in leaves and seeds' crude methanolic extracts but the seeds exhibited powerful anthelmintic action as compared to leaves, therefore, it is concluded that this vegetable, *Portulaca oleracea*, can be used as potential medicine for possible removal of worm, *Lumbricus terrestris*, from human intestine. No such research work has been reported on this plant prior to us.

In Traditional Chinese Medicine, it is stated that, "it is used to treat infections or bleeding of the genito-urinary tract as well as dysentery. The fresh herb may also be applied topically to relieve sores and insect or snake bites on the skin (Bensky *et al.*, 2004). Eating purslane can dramatically reduce oral lichen planus" (Agha-Hosseini *et al.*, 2010).

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