

Research Article

In-vitro Anthelmintic Activity of Leaves of *Spinacia oleracea* Linn.

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ABSTRACT

The present study was undertaken to evaluate anthelmintic activity of crude extract of *Spinacia oleracea* Linn. and different extract namely fresh juice extract, petroleum ether extract and methanolic extract using *Pheretima posthuma* as test worms. Different concentration 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml of fresh juice extract and methanolic extract of *Spinacia oleracea* Linn. Each extract was studied which determined time of paralysis and time of death of worms. Both the extract performed invitro anthelmintic activity. Albendazole was used as standard reference and saline water as control. The result of present study revealed that the fresh juice extract may show more potent anthelmintic activity than methanolic extract. Further studies are in process to isolate the active principle responsible for the activity.

Keywords: Anthelmintic activity, *Spinacia oleracea*.

INTRODUCTION

Parasites have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance.^[2] There are various plants which are reported as anthelmintic such as *Pongamia glabra*,^[11] *Enhyra fluctuans*,^[12] *Mimusops Elongi linn*,^[13] *Mentha Piperita*,^[14] *Lantana Camara*,^[14] *Picrolemma Sprucei hook f.*,^[18] *Trachyspermum Ammi*,^[16] *Nigella Sativa*,^[18] *Azadirachta Indica*,^[19] *Clitoria Termatea linn.*,^[20] *Terminalia chebula*,^[19] and many other plants. *Spinacia oleracea* Linn which is commonly known as spinach belonging to family as Amaranthaceae and its native to central and southwestern Asia. It has been used medicinally in treat of anemia, nightblindness, tooth disorder, urinary disorder, cancer, and respiratory disorder. It is also used as antioxidant, anti-ageing agent, sun protective, antipyretic agent and anti inflammatory.^[3]

Spinach contains a number of antioxidants including carotenoids, polyphenols, and flavonoids (quercetin). The carotenoids are composed of 2 main classes, carotenes (betacarotene) and xanthophylls (lutein). Glycolipids (sulfoquinovosyl diacylglycerol) are found in the chloroplast membrane. Spinach is regarded as a valuable dietary source

of vitamin A, nonheme iron, folate, and lutein. It contains a number of antioxidants including carotenoids, polyphenols, and flavonoids. Spinach is regarded as a valuable dietary source of vitamin A, non heme iron, folate, and lutein.^[4] Literature search revealed that there is no scientific report available regarding anthelmintic activity of *Spinacia oleracea* Linn leaves.

MATERIALS AND METHOD

Plant materials

Leaves of *Spinacia oleracea* Linn. was collected from open areas of Bhopal (Madhya Pradesh) during winter seasons which is very suitable and fully developed leaves and authenticated by Dr. Madhavi Modak, Department of Botany, MVM college, Bhopal (M.P.)

Preparation of extracts

Preparation of Fresh juice extract^[2,1]

Collected leaves were weighed (75g) and blended into liquefactions in 150ml of water. The mixture was then centrifuged at 150 rpm. The supernatant was filtered through sterile filter paper in to conical flask as the study extract 1ml of filtrate is expected to contain 0.5g i.e. 500mg/ml

Preparation of methanolic extract

To remove fatty material from extract of weighed powdered leaves are defatted with petroleum ether at 60-80°C for 72 hrs in Soxhlet apparatus. Complete defatting of drug is ensured by placing a drop from the thimble on a filter paper give any oily spot. The marc was dried in air to remove traces of petroleum ether. The defatted plant material was again subjected to hot continuous extraction with 95% of

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methanol in Soxhlet apparatus. The extract was dried and stored in dark place.

polyphenolic compounds.^[8] It is possible that tannins contained in the extracts of *Spinacia oleracea* Linn

Table:1 Invitro Anthelmintic activity of *Spinacia oleracea*

Test Samples	Conc. mg/ml	Time taken For Paralysis (minutes)	Time taken for Death (minutes)
Fresh juice extract	10	29 ± 0.36*	37.6 ± 0.20*
	20	11.5 ± 0.22*	26.1 ± 0.16*
	30	9.6 ± 0.21**	17.6 ± 0.21*
	40	8.3 ± 0.21*	15.60 ± 0.21**
	50	6.5 ± 0.22*	14.16 ± 0.16*
Methanolic extract	10	27.2 ± 0.37*	35 ± 0.25*
	20	10.3 ± 0.33**	24.83 ± 0.16*
	30	8.1 ± 0.30*	15.83 ± 0.16**
	40	5.6 ± 0.21*	14.6 ± 0.21*
	50	4.8 ± 0.16*	12.6 ± 0.21*
Albendazole	20	10 ± 0.25*	15.83 ± 0.30*

All values represent Mean ± SEM; Values are significantly different from reference standard (Albendazole) where *P<0.01, **P<0.05 Vs Albendazole, SEM:Standard error mean, n=Number of animals in each groups (6)

Drug and Chemicals

Albendazole, methanol, DMF and Saline water.

Animal

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of Chambal fertilizer and chemical Ltd. M.P. Nagar star Arcade zone-1, Bhopal. Earthworms were washed with normal saline to remove all faecal matter, were used for anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

Anthelmintic Activity

Fresh juice extract and methanolic extract of leaves of *Spinacia oleracea* Linn. were dissolved in minimum amount of DMF and the volume was adjusted to 10ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. In each case, 6 earthworms were released into 10ml of desired formulations as follows: vehicle (5%DMF in normal saline), Albendazole (20mg/ml), or fresh juice extract and methanolic extract of leaves of *Spinacia oleracea* Linn. In normal saline solution containing 5% DMF. Observation was made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in saline solution.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of methanolic extract of *Spinacia oleracea* Linn showed the presence of cyogenetic glycosides, protein, amino acid, carbohydrates, flavanoids, phenol and tannins whereas fresh juice extract contains alkaloids, cyogenetic glycosides, protein, amino acid, carbohydrates, flavanoids, phenol and tannin. It is evident from experimental data that methanolic extract and fresh juice extract showed significant activity.

As shown in table 1, fresh juice extract and methanolic extract in dose dependent manner showed anthelmintic activity. Result were comparable with standard drug, Albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities^[7] in the invitro anthelmintic activities of *Clitoria Ternatea* linn.,^[20] *Enhydra fluctuans*,^[12] and *Mimusops Elongi* linn.^[13] Chemically tannins are

produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal^[9] or glycoprotein on the cuticle of the parasite^[10] and may cause death.

In conclusion, the traditional use of leaves of *Spinacia oleracea* Linn as an anthelmintic has been confirmed as the leaves extract displayed profound anthelmintic activity in the study. Further it would be interesting to isolate the possible phytoconstituent and characterize the active constituents which may be possible responsible for the anthelmintic activity and to possible the mechanism of action.

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