SHORT COMMUNICATION

In Vitro Anthelmintic Activity of *Achyranthes aspera* Linn. (Whole Plant) Against *Pheretima posthuma*

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ABSTRACT

Introduction: Helminthiasis or worm infestation is one of the most prevalent diseases in the world. It is one of the most serious problems in the developing countries. Anthelmintic plants are used traditionally to treat parasitic infections. *Achyranthes aspera (Amaranthaceae*) is an important medicinal plant found as a weed throughout India and tropical Asia. The aim of the present study was to evaluate the anthelmintic activity of *A. aspera* against *Pheretima posthuma* (earthworm). **Methods:** The anthelmintic activity was evaluated on adult Indian earthworms by reported methods. **Results:** The anthelmintic activity was studied using ethyl acetate, ethanol and aqueous extracts of the whole plant at a test concentration of 25, 50, 75 and 100 mg/ml each. Albendazole was used as standard. All the three extracts have exhibited significant dose dependent anthelmintic activity at the tested dose level. Aqueous extract was found to have better activity followed by ethyl acetate extract and the least activity was reported for ethanol extract. **Conclusion:** Thus from the present study, it can be concluded that *A.aspera* extracts (Aqueous>Ethyl acetate> Ethanol) have potent anthelmintic activity when compared with the conventional drug, albendazole. Further studies are required to isolate the active principles and also to establish the pharmacological rationale for the anthelmintic activity of *A. aspera*.

KEY WORDS

Achyranthes aspera, anthelmintic activity, earthworm, helminthiasis, medicinal plants, worm infestation.

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world.[1] Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anemia and pneumonia.[2] Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. There are numerous species of these parasites, which are broadly classified into tape worms, flukes, and roundworms.[3] They often live in the gastrointestinal tract of their hosts, but they may also burrow into other organs where they induce physiological damage. Anthelmintic drugs (albendazole, mebendazole, praziquantel, ivermectin and piperazine) are a group of anti-parasites that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them without causing significant damage to the host. The gastro-intestinal helminths becomes resistant to currently available anthelmintic drugs therefore

there is a foremost problem in treatment of helminths diseases. Hence there is an increasing demand towards natural anthelmintics.[4] As the half of world suffering from bacterial and helminths infection, the source of infection being very common due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions.[5]

Herbal medicine is still the main source of medicine and about 75-80% of the whole population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility, with the human body and fewer side effects. Evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days.

Achyranthes aspera Linn. is one of the numerous medicinal plant species with remarkable therapeutic potential that is commonly recognized as Prickly Chaff flower (English). In Tamil it is called as *Chirukadaladi, Naayurivi*. The species belongs to the *Amaranthaceae* family and is widely distributed as a weed throughout the tropical and subtropical regions of the world.[6] The plant is popular in folk remedy in traditional systems of medicine in tropical Asia and African countries. Its diverse uses in the various traditional healthcare systems include the treatment of fever, wound healing, tooth ache, arthritis, gynaecological disorders, urinary disorders, insect and snake bites, abdominal tumours, stomach pain and a number of other ailments.[7] The plant is reported to be used as antimicrobial, larvicidal, antifertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, anti-anasacra, analgesic, antipyretic, antinoiceptive, prothyrodic and antispasmodic activity.[8]

Though *A.aspera* was explored for many of its pharmacological activity, there were no reports on the anthelmintic activity of the selected plant with respect to standard. Hence, the present study was aimed at to evaluate the antihelmintic activity of various extracts of *Achyranthes aspera* (whole plant) against *Pheretima posthuma,* the earthworm.

The fresh and healthy whole plant of *A. aspera* were collected from Guduvanchery, Tamilnadu. The plant was authenticated by Prof. Dr. P. Jayaraman, Ph.D., Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai - 45.

The collected plant material was washed with water. It was then chopped into small pieces, dried at room temperature for 7 days and grinded into coarse powder with a mechanical grinder and stored in an airtight container. 20g powder was macerated in 100ml of 95% ethyl acetate, 100ml of water and in 100 ml of 95% ethanol, separately for 3 days at room temperature with occasional stirring. After 3 days, ethyl acetate, aqueous and ethanol extract was filtered through Whatman No.1 filter paper. The filtrate obtained was concentrated under reduced pressure through distillation. The resultant extract was suspended in normal saline containing Tween 20 (1%) and used for anthelmintic activity.

Healthy adult Indian earthworms, *Pheretima posthuma (Annelida, Megescolecidae)* were used for evaluating the anthelmintic activity due to its anatomical and physiological resemblance with intestinal round worm parasites of human beings. [9-10] All healthy earthworms were of approximately 8-10cmin size. They were collected from Guduvanchery, Chennai washed and kept in water until they are used for screening of activity.

The anthelmintic activity was evaluated on adult Indian earthworms by reported methods. [9-10] The sample extracts were prepared at the concentration of 25, 50, 75, 100 mg/ml in Tween 20 (1%) solution diluted with normal saline. six worms (*Pheretima posthuma)* of 8-10cm were placed in petri dish containing 30 ml of above test extracts. Albendazole (25, 50, 75, 100 mg/ml) was used as reference

standard. All the test solutions and standard solutions were prepared freshly before starting the experiment.

Observations are made for the time taken for paralysis when movement was lost or no movement. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water and fading of color of worms. [11]

Plants have been utilized as a natural source of medicines since thousands of years in Siddha, Ayurveda, Unani, and other traditional medical systems practiced worldwide. Traditional Indian literatures also reported the use of medicinal plants to antidote human diseases in ancient times. Currently, scientists are focusing on the active compound from the natural sources to develop newer drugs. The present study has investigated the anthelmintic activity of whole plant extract of *A. aspera* (aqueous, ethyl acetate and ethanol) by *in vitro* methods.

From the results, it was evident that the earthworms lost their motility on exposure to aqueous, ethyl acetate and ethanol extract of *A. aspera*. All the three extracts has shown dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, at the tested concentration of about 25, 50, 75 and 100 mg/ml respectively.

Among the three extracts, aqueous extract has shown greater activity when compared with the two other extracts (ethyl acetate and ethanol). Aqueous extract could produce paralysis within 35.34, 26.13, 17.19 and 15.42 min at 25, 50, 75 and 100mg/ml respectively. Ethyl acetate extract has produced paralysis within 45.12, 32.37, 30.25 and 35.51 min at 25, 50, 75 and 100 mg/ml respectively. Ethanol extract has shown lesser activity when compared with the other two extracts. It could produce paralysis at 59.44, 50.38, 40.15 and 28.34 min at 25, 50, 75 and 100mg/ml respectively. The standard drug albendazole has caused paralysis within 23.51, 20.52, 13.1 and 10.40 at 25, 50, 75 and 100 mg/ml respectively (Table 1).

Table 1. Anthelmintic activity of *Achyranthes aspera* extracts on earthworms

Drug	Concentration mg/ml	Time taken for paralysis (min)	Time taken for death (min)
Ethyl acetate	25	45.12	57.24
	50	32.37	42.39
	75	30.25	37.29
	100	35.51	38.51
Ethanol	25	59.44	71.34
	50	50.38	62.12
	75	40.15	52.34
	100	28.34	37.14
Aqueous	25	35.34	49.37
	50	26.13	38.16
	75	17.19	26.32
	100	15.42	20.29
Albendazole	25	23.51	45.14
	50	20.52	40.36
	75	13.1	32.27
	100	10.40	26.51

Paralysis eventually progressed to death of the earthworms. Mortality was produced at 49.37, 38.16, 26.32 and 20.29 min by the tested concentration 25, 50, 75 and 100mg/ml of aqueous extract of *A. aspera*. Ethyl acetate extract has caused death at 57.24, 42.39, 37.29 and 38.51 min by the tested concentration 25, 50, 75 and 100mg/ml respectively. Longer time was taken by the ethanol extract of *A. aspera* to cause death of the earthworms. 25, 50, 75 and 100mg/ml of ethanol extract causes death at 71.34, 62.12, 52.34 and 37.14 min respectively. The standard drug albendazole has caused death at 45.14, 40.36, 32.27 and 26.51 min at 25, 50, 75 and 100 mg/ml respectively. From the above observations, it is evident that all the three extracts of *A. aspera* have anthelmintic activity when compared to the standard drug. The order of anthelmintic activity was found to be aqueous>ethyl acetate>ethanol extract and

the aqueous extract results were near to the standard drug.

Many researchers have reported the presence of phytochemicals like flavonoids, phenols, steroids, tannins, terpenoids, glycosides and coumarins in various parts of *A. aspera*.[6,12-15] The present investigation was done using the whole plant extract. Hence, the anthelmintic activity of the selected medicinal plant might be due to the presence of these phytochemicals. These phytochemicals may follow different mechanism to cause death of the earthworm. Jadhav and Gharvate *et al*[16] has reported that tannins present in the ethanol extract of *A. aspera* stem can bind to free proteins in the gastrointestinal track of the host animal or glycoproteins on the cuticle of the parasite and thereby causes death of the earthworm. Similar kind of mechanism may be responsible for the anthelmintic activity observed in the study.

From the present study, it can be concluded that the whole plant extracts of *A. aspera* (aqueous, ethyl acetate and ethanol) has potent anthelmintic activity when compared with the standard drug, albendazole. Further studies using animal models are required to establish the pharmacological rationale for the same. Besides, identification and isolation of active principles responsible for this activity has to be done to formulate the extract into suitable dosage forms.

Conflict of Interest

The authors declare no conflict of interest.

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