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Research Article

ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF STEMS OF *Achyranthus aspera* Linn.

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ABSTRACT

The present study was undertaken to evaluate the anthelmintic activity of leaves of *Achyranthes aspera* Linn. (Amaranthaceae), in a scientific manner. All parts of plant have been reported to relieve a variety of ailments. The anthelmintic activity was evaluated on adult Indian earthworms *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being. The ethanol extract of stem of *Achyranthes aspera* (EEAA) at 10, 25 and 50 mg/ml caused paralysis followed by death of worms at all tested dose level with standard as Albendazole. The EEAA exhibited significant anthelmintic activity at concentration of 10, 25 and 50 mg/ml as compared to reference standard Albendazole. The potency of extract was found inversely proportional to time taken for paralysis / death of worms.

Key words: *Achyranthes aspera* Linn., *Pheretima posthuma*, Anthelmintic activity, Ethanolic extract, Albendazole.

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INTRODUCTION

Helminthiasis or infection with parasitic worms are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better known conditions i.e., tuberculosis and malaria (Silva NR *et al.*, 2003). The disease is highly prevalent particularly in third world countries due to poor management practices (Dhar DN *et al.*, 1982). In tropical regions, where prevalence is greatest, simultaneous infection with more than one type of helminths is common. Moreover, human beings can spread these pathogens to previously uninvolved populations through travel, migration and military operations. There are two major phyla of helminthes of which the nematodes includes the major intestinal worms and filarial worms that cause lymphatic filariasis and onchocerciasis, whereas the Platyhelminthes include the flukes, such as the schistosomes and the tapeworms, such as the pork tapeworm that causes cysticercosis(Kumar VK *et al.*, 2004). The World Health Organization reveals that over two billion people are suffering from parasitic worm infections (Hotez PJ *et al.*, 2008). It is estimated that by the year 2025, about 57% of the population in the developing countries will be influenced by helminths infections (Gunaselvi G *et al.*, 2010).

In recent times focus on plant research has increased all over the world and there is widespread of belief that the green medicines are healthier and harmless than the synthetic ones (Murthy KNC *et al.*, 2005). Plants are good sources for new, safe, biodegradable and renewable drugs however the use of plants as therapeutic agents in addition to being used as food is age long(Joy V *et al.*, 2012).

Anthelmintic plants are used traditionally in expelling the worms that is parasitic in nature from the

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body either by stunning or killing them. They are also known as Vermifuges or Vermicides (Prakash V et al., 2008). Anthelmintic medicinal plants are used as good alternatives for the traditional allopathic medicinal agents.

Achyranthes aspera Linn. (Family-Amaranthaceae) is an important medicinal herb found as a weed throughout India and tropical Asia. It is known by different names such as Apamarga(Sanskrit), Latjeera (Hindi), Aghada(Marathi) and Rough Chaff Tree (English). It is an erect or procumbent, annual or perennial herb, 1 to 2 meters in height, often with a woody base commonly found as a weed of waysides, on roadsides (Dastur RH 1925). Traditionally, the plant is used in asthma. It has various medicinal properties reported such as cardiovascular agent, pungent, antiasthmatic, astringent and diuretic and as a spermicidal. Plant also used as abortifacient, antibacterial and antifungal, anti-allergic, chemo protective, antiemetic. This plant was reported to contain saponins A and B (HariharanV and RangaswamiS, 1970; Ali M, 1993). Saponin A was identified as D-glucuronic acid and Saponin B was identified as β-D- galactopyranosyl ester. This plant was reported to contain saponins A and B. Saponin A was identified as D-glucuronic acid and Saponin B was identified as β-D-galactopyranosyl ester of D-glucuronic acid. Along these other constituents include oleanolic acid, hentriaconatase amino acids (Saurabh S *et al.,* 2001). There were no reports on the anthelmintic activity of *Achyranthes aspera* Linn. stem extracts, only few reports were found on leaf extracts. This promoted us to investigate the anthelmintic activity of ethanolic extracts of stems of *Achyranthes aspera* Linn. with respect to standard drug Albendazole.

MATERIALS AND METHODS

The stems of the plant *Acyranthus aspera* was collected from the local areas of Ahmednagar District (MPKV, Rahuri) and were authenticated by Botanical Survey of India Pune, Maharashtra, India.(Voucher specimen No. BSI/WRC/IDEN.CER.2016-17.SSW05). Prepared herbarium was deposited at Department of Pharmacognosy, MES's College of Pharmacy, Sonai.

Extraction

The stems were dried under shade, reduced to moderately coarse powder, loaded into Soxhlet extractor and were subjected to successive extraction with ethyl alcohol (90%). A total of 50 cycles were run to obtain thick slurry. This slurry was then vaccum evaporated to yield a solid extract. The dried extract was stored in a well closed, air tight and light resistant borosil glass container.

Preliminary Phytochemical Screening

In order to determine presence of various phytoconstituents, a preliminary phytochemical study with plant extract was performed (Khandelwal K R, 2002).

DRUGS AND CHEMICALS

Albendazole, ethanol (Qualigen Fine Chemicals, Mumbai).

Anthelmintic Bioassay

The earthworm *Pheretima posthuma* (Annelida, Megescolecidae) was used for evaluating the anthelmintic activity of crude extract using reference substance for comparison. These were procured from local supplier and maintained at MES's College of Pharmacy, Sonai (Vidyarthi RD, 1965; Chatterjee KD, 1967; Dash GK *et al.,* 2002)

Evaluation of Anthelmintic Activity

Anthelmintic activity was assessed using earthworms by the reported methods with slight modification. Samples for anthelmintic activity were prepared by dissolving 2.5 gm dried crude extracts in 25 mL 1% gum acacia solution prepared in normal saline (vehicle). To obtain a stock solution, different working solutions were prepared to get a concentration range of 10, 25 and 50 mg/mL. The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being. The activity of crude ethanolic extracts of stems of *Achyranthes aspera* Linn was determined by using five groups of approximately equal size Indian earthworms consisting of six earthworms in each group were used for the study. Each group was treated with one of the following.

Group-I - Control (1% Gum acacia in normal saline)
Group-II - Albendazole (25 mg/ml)
Group-III, IV, V - Extracts (10, 25 and 50 mg/ml.)

Observations were made for the time taken to paralyze and / or death of individual worms. Paralysis was said to occur when the worms do not move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour (Chatterjee KD, 1967; Dash GK *et al.,* 2002).

Statistical Analysis

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered significant.

Table 1. Anthelmintic activity of ethanolic extract of stems of *Achyranthus aspera*

S. N.	Groups	Dose mg/ ml	Time of paralysis(min.) ± SEM	Time of death(min) ± SEM
1	I. Control	---	---	---
2	II. Extract	10	3.59 ± 0.23*	5.20 ± 0.04*
3	III Extract	25	2.94 ± 0.21**	4.39 ± 0.06*
4	IV Extract	50	2.68 ± 0.23**	3.19 ± 0.04**
5	VI Albendazole	25	1.50 ± 0.03	1.50 ± 0.04

Values are mean ± SEM, n=6. When compared with Control using one way ANOVA followed by Dunnett's multiple comparison test.

RESULTS

Preliminary phytochemical screening of the ethanolic extract of stem showed the presence of glycosides, phenols, coumarins, tannins, flavanoids, steroids and terpenoids. Anthelmintic activity of ethanolic extract of stems of *A. aspera* was evaluated. From the observations made when compared to the standard drug Albendazole (Table 1), the earthworms lost their motility on exposure to ethanolic extract of stems of A. aspera. Each ethanolic dose 10, 25 and 50 mg/ml concentration produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progresses to death. The concentration 10, 25 and 50 mg/ ml of extract produced paralysis within 3.59, 2.94 and 2.68 min. respectively when compared with standard drug Albendazole which causes paralysis within 2.46,1.50 and 0.51 minutes.

Mortality was produced by concentration 10, 25 and 50 mg/ ml of extract within5.29, 4.39 and 3.19 min. respectively when compared with standard drug Albendazole which causes death within 3.29, 1.50and 1.18 min. respectively. Haemorrhagic and necrotic spots were observed externally on the worms, with the higher concentration.

DISCUSSION

Anthelmintic activity of the ethanolic extract of stems of A*chyranthus aspera* was tested by using Indian adult earthworms *Pheretima posthuma.* Phytochemical analysis of the crude drug extract reveals presence of tannins and phenolics as one of the chemical constituents. Tannins were shown to produce anthelmintic activity (Mali RG and Wadekar RR, 2008; Subrat K *et al.*, 2008; Mahajan SG *et al.*, 2008). Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics like niclosamide, oxyclonazide and bithionol are shown to interfere with energy generation in helminths parasites by uncoupling oxidative phosphorylation. It is possible that tannins present in ethanolic extract of stems of *A. aspera* produces similar effect.

Another possible anthelmintic effect of tannins is that, they can bind to free proteins in the gastrointestinal track of the host animal or glycoproteins on the cuticle of parasite and thereby causes death.

CONCLUSION

From the present work, it can be concluded that the ethanolic extract of the stems of *A. aspera* have potent anthelmintic activity when compared with the conventionally used drugs. Further studies using in vivo models are required to carry out and establish the pharmacological rationale for the use of *Achyranthes aspera* as an anthelmintic drug. Apart from this further studies are necessary to isolate the active principles and to formulate the extract into suitable dosage forms.

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CONFLICT OF INTEREST

No interest

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