

# ***In-vitro* Anthelmintic Activity of Methanolic and Aqueous Extracts of *Achyranthes aspera* linn. (Amaranthaceae) Stems**

Naga Bharathi. M\*, Sravanthi. V, Sujeeth. S, Kalpana. K, Santhoshi. P, Pavani. M, Rajan Kumar Singh and P. Uma Devi

Viswanadha Institute of Pharmaceutical Sciences, Mindivanipalem, Sontyam, Anandapuram (M), Visakhapatnam, India

\* Corresponding author: Naga Bharathi; e-mail: [bharathimarni@gmail.com](mailto:bharathimarni@gmail.com)

Received: 12 February 2013

Accepted: 01 March 2013

Online: 05 March 2013

## **ABSTRACT**

The Anthelmintic activities of methanolic and aqueous extracts of stems of *Achyranthes aspera* linn. were evaluated separately on Indian adult earthworms *Pheretima posthuma* (Annelida). For this work the stems were extracted separately with methanol and distilled water by following maceration method. Various concentrations (2.5, 5, 10, 20 mg/ml) of each extract were tested for anthelmintic activity which involved the determination of time of paralysis and time of death of the worms. The phytochemical screening of the crude extract showed the presence of saponins A and B, terpenoids, volatile oils. All extracts were able to show anthelmintic activity at all concentrations. The activities are well comparable with the standard drug, Albendazole as positive control. All the methanolic extracts showed better anthelmintic activity than the standard drug as well as the aqueous extract. Tween 20 (1%) with saline was used as negative control, did not showed any anthelmintic activity.

**Keywords:** *Achyranthes aspera* linn., *Pheretima posthuma*, Anthelmintic activity, Methanolic extract, Aqueous extract

## **INTRODUCTION**

Helminthiasis or worm infestation is one of the most prevalent disease and one of the most serious problems in the world. It contributes to malnutrition anemia, eosinophilia and pneumonia [1, 2]. A number of plants have been tested for their anthelmintic efficacy [3]. *Achyranthes aspera* linn. is known as uttareni. It belongs to family Amaranthaceae [4]. Due to limited availability and affordability of pharmaceutical medicines, species of higher plants are used for the treatment of helminthiasis. According to W.H.O. more than 80% of the world's population relies on medicinal plants [1, 3]. *Achyranthes aspera* linn. is an important medicinal herb found as a weed throughout India. It is known by different names such as apamarga (Sanskrit), latjeera (Hindi) and rough chaff tree (English). It is an erect or procumbent, annual or perennial herb, 1 to 2 meters in height, often with a broody base commonly found as a weed of waysides, on roadsides [5, 6, 7]. This plant was reported to contain saponins A and B [8, 9]. Saponin A was identified as D-glucuronic acid and Saponin B was identified as  $\beta$ -D-galactopyranosyl ester

of D-glucuronic acid. Along these other constituents include oleanolic acid, hentriaconatase amino acids [4]. 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 and compare the anthelmintic activity of aqueous and methanolic extracts of stems of *Achyranthes aspera* linn. with respect to standard.



*Achyranthes aspera* linn.

## **Traditional uses:**

Traditionally, the plant is used in asthma. It has various medicinal properties reported such as cardiovascular

agent [14], pungent, antiasthmatic, astringent and diuretic and as a spermicidal [15], abortifacient, antibacterial and antifungal, anti allergic, chemo protective, antiemetic.

## MATERIALS AND METHODS

### Drugs and chemicals:

Albendazole (Gift sample obtained from Vera's pharmaceuticals, A.P), Methanol (Merck Pvt. Mumbai) and other chemicals were procured from suppliers.

### Plant material:

The plant *Achyranthes aspera* linn. was collected from Mindivanipalem village, Visakhapatnam district, Andhra Pradesh during the month of November 2012. The plant material was identified and authenticated by Dr. M.V.R.K. Narasimhacharyulu, MSc, M. Phil, PhD, Principal of Sri Y.N.College of botany, Narasapuram, West Godavari district, A.P. The collected stems were shade dried under normal environmental conditions, powdered, stored at 4-6°C in refrigerator, in a closed container for further use.

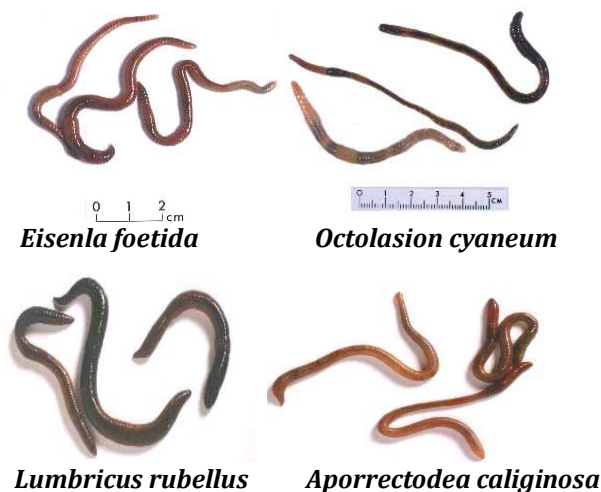
### Preparation of extract:

The powdered stems were passed through a sieve (No.60) and then those stems (40 gm) of *Achyranthes aspera* linn., were extracted by using maceration method. The powdered stems were macerated in 400 ml of methanol for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness by distillation process. The dried extracts (residue) were suspended in normal saline containing Tween 20 (1%) and used for screening the anthelmintic activity.

### Animals:

Healthy adult Indian earthworms *Pheretima posthuma* were used for evaluating the anthelmintic activity due to its anatomical and physiological resemblance with intestinal round worm parasites of human beings [2].

Different types of worms causing helminthiasis [10]



All healthy earthworms were of approximately 5-7 cm in size. They were collected from local place, washed

and kept in water until they are used for screening of activity.

### Anthelmintic activity

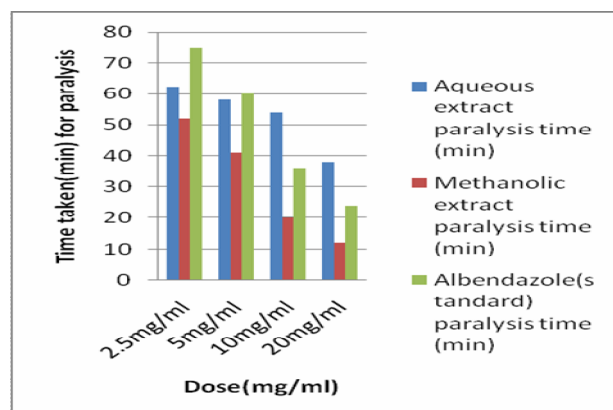
The anthelmintic activity was evaluated on adult Indian earthworms by Mathew *et.al* method[1]. For preliminary evaluation of anthelmintic activity test samples of the extract was prepared at the concentration of 2.5, 5, 10, 20 mg/ml in Tween 20 (1%) solution diluted with normal saline and 6 worms *Pheretima posthuma* of 8-10cm were placed in petridish containing 30 ml of above test solutions of extracts. Albendazole (2.5, 5, 10, 20 mg/ml) was used as reference standard and normal saline with Tween 20 (1%) is used as negative control. 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. Worms should not relieve even in normal saline. 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.

## RESULTS AND DISCUSSION

The results in the Table-1 depict the time taken for paralysis after treating with the test substances.

**Table 1.** Anthelmintic activity (paralysis) of *Achyranthes aspera* linn. stems

Type of extract	Dose (mg/ml)	Time (min) taken for paralysis of earthworms
Aqueous	2.5	62
	5	58
	10	54
	20	34
Methanolic	2.5	52
	5	41
	10	20
	20	12
Albendazole (positive control)	2.5	75
	5	60
	10	36
	20	24
Vehicle (1% Tween 20)	-	-

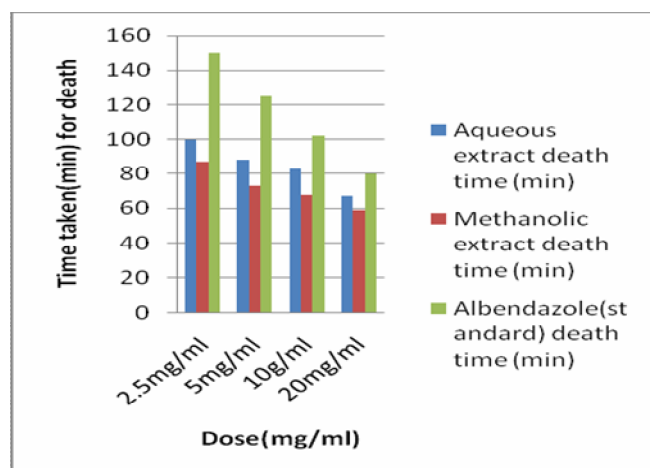


**Figure 1.** Anthelmintic activity (paralysis) of aqueous and methanolic extracts of *Achyranthes aspera* linn. stems

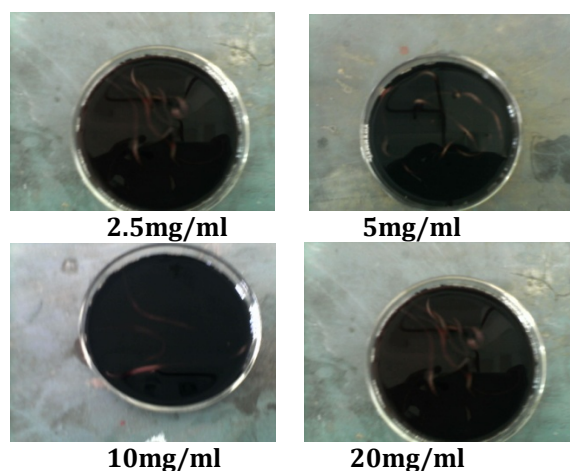
The results in the Table-2 depict the time taken for death after treating with the test substances.

**Table 2.** Anthelmintic activity (death) of *Achyranthes aspera* linn. stems

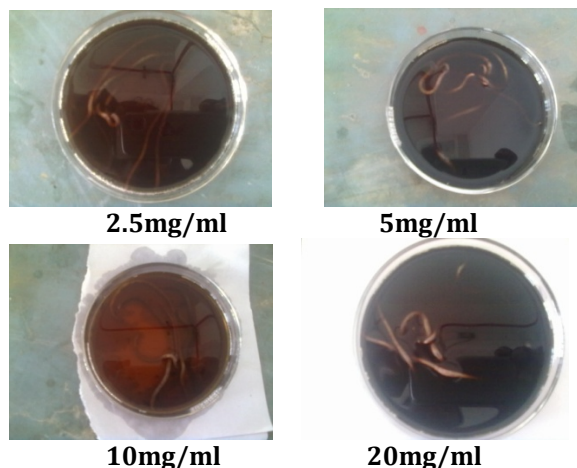
Type of extract	Dose (mg/ml)	Time (min) taken for paralysis of earthworms
Aqueous	2.5	100
	5	88
	10	83
	20	67
Methanolic	2.5	87
	5	73
	10	68
	20	59
Albendazole (positive control)	2.5	150
	5	125
	10	102
	20	80
Vehicle (1% Tween 20)	-	-



**Figure 2.** Anthelmintic activity [death] of aqueous and methanolic extracts of *Achyranthes aspera* linn. stems



**Figure 3.** Anthelmintic activity (paralysis) of methanolic extracts of *Achyranthes aspera* linn. stems



**Figure 4.** Anthelmintic activity (death) of aqueous extracts of *Achyranthes aspera* linn. stems

From the literature, it was found that shoots contain various medicinally important chemical constituents such as aliphatic dihydroxyketone, 36, 37-dihydroxyheptacosan-4-on, Triacontanol, 27-cyclohexylheptacosan-7-ol and 16-hydroxy 26-methyheptacosan-2-on [11, 12]. The Petroleum ether extract of the shoots produced a yellow semi-solid mass. From this a pink colored essential oil with pleasant odor and an aliphatic alcohol (17-pentatriacontanol) was found to possess antifungal activity [13]. Anthelmintic activities of different extracts were evaluated. Extract at a concentration of 20 mg/ml, produced paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death. The aqueous extract of stem of *Achyranthes aspera* linn. at high concentration showed good anthelmintic activity. The methanolic extracts of *Achyranthes aspera* linn. stem extract at normal concentration only showed good anthelmintic activity and this is compared with the effect produced by the reference standard drug, Albendazole. The results in Table-1 and Table-2 indicate that Aqueous and Methanolic extracts obtained from the stems of *Achyranthes aspera* linn. are active against the *Pheretima posthuma* tested. It is worth to mention that the stem methanolic extracts are comparatively more active than Albendazole against *Pheretima posthuma*. These findings support the use of *Achyranthes aspera* linn. as anthelmintics in the traditional medicine. The present study reveals that the methanolic extract was more potent than the aqueous extract even though both the extracts were endowed with anthelmintic activity. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

## CONCLUSION

In conclusion, it is revealed that the Aqueous and Methanolic extracts obtained from the stems of *Achyranthes aspera* linn. possess anthelmintic activity, but the methanolic extract showed enhanced anthelmintic activity when compared with the aqueous extract and standard drug, Albendazole. The active

constituents such as aliphatic dihydroxyketone, 36, 37-dihydroxyhenpentacontan-4-on and Triacontanol might be responsible for anthelmintic activity because of their neurotoxin action. In order to confirm the above results, the *in vivo* studies have to be conducted.

#### ACKNOWLEDGEMENTS

The author M. Nagabharathi, Assistant Professor is thankful to the management of Viswanadha Institute of Pharmaceutical Sciences, Sontyam, Anandapuram (M), Visakhapatnam, India for providing the laboratory facilities to carry out this work.

#### REFERENCES

1. D. Anantha et al., Invitro anti helmentic activity of aqueous and alcoholic extracts of aerva lanata seeds and leaves, *J. Pharmaceutical sciences and research*, **2010**, 2 (5), 317-321.
2. Choudhury Golak Bihari et al., Phytochemical investigation and screening of anthelmintic activity of leafy extracts of various Ocimum (Tulsi) species, *J. Pharmaceutical sciences and research*, **2010**, 3 (9), 2140-2141.
3. Bushan Maley et al., Anthelmintic activity of ethanolic and aqueous extracts of *Momordica charantia* (curcubitaceae), *International journal of herbal drug research*, **2012**, 1 (4), 10-13.
4. Saurabh Srivastav et al., *Achyranthes aspera*- An important medicinal plant: A review , *J. Nat. Prod. Plant Resour.* **2011**, 1 (1), 1-14.
5. Jitendra B. Jain, Sheetal C. Kumane, S Bhattacharya. Medicinal flora of Madhya Pradesh and Chattisgarh – A Review *Indian journal of Traditional Knowledge*, **2006**, 5 (2), 237-242.
6. Anonymous. *The wealth of India – Raw Materials*, Council of Scientific & Industrial Research, New Delhi, **2005**, 55-57.
7. R. Zafar. *Medicinal Plants of India*. CBS publishers & distributors, **2009**, 1-15.
8. Hariharan, V. ; Rangaswami, S., Structure of saponins A and B from the seeds of *achyranthes aspera* *Phytochemistry*, **1970** 9 (2). pp. 409-414.
9. M. Ali. , chemical investigation of *Achyranthes aspera* Linn. *Oriental journal of Chemistry*, **1993**, 9 (1), 84-85.
10. T. Shri Vijaya Kirubha, Anthelmintic activity of roots of rhizomes of *Corallocarpus Epigaeus* *J. Nat. Prod. Plant Resour.* **2011**, 1 (1), 81-84.
11. Batta AK, Rangaswami S. Crystalline chemical components of some vegetable drugs *Phytochemistry* **12**, **1973**, 214-6.
12. T. G. Misra, R. S. Singh, H. S. Pandey. Two long chain compounds from *Achyranthes aspera*., *Phytochemistry*, **1993**, 33(1), 221-223.
13. Y. Gariballa, G.M. Iskander, El Beit Daw , A Investigation of the alkaloid components in the Sudan Flora., *Fitoterapia*, **1983**, 54, 269-272.
14. N. C. Neogi, R. D. Garg, R. S. Rathor. Preliminary pharmacological studies on *achyranthine*, *Indian journal of Pharmacy*, 1970, 32 (2), 43-46.
15. D. Paul, D. De, K. M. Ali, K. Chatterjee, D. K. Nandi, D. Ghosh. *Contraception*, **2010**, 81 (4), 355-361

© 2013; AIZEON Publishers; All Rights Reserved

This is an Open Access article distributed under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

\*\*\*\*\*