An Promising Anthelmintic Activity: Comparative Studies, Chemical Analysis and Characterization of Different Species of Orgnic Extracts of *Achyranthes Linn*.

Loganathan M*, Saravanakumar A, Parthiban P, Dhayanitha S, Madhumitha R, Deepasri R and Chitra V

Department of Pharmaceutical Chemistry, Vellalar College of Pharmacy, Maruthi Nagar, Thindal Post, Erode 638012, Tamilnadu, India

ABSTRACT

Helminthiasis (worm infestation) is one of the most prevalent infectious diseases worldwide, particularly across developing regions. Medicinal herbs are traditionally used to manage helminthic disorders due to their cost-effectiveness and availability. *Achyranthes aspera* and *Achyranthes bidentata* belonging to the family Amaranthaceae are ethnomedicinally known for their anthelmintic properties. The present study investigates the anthelmintic activity of whole-plant extracts of *A. aspera* and *A. bidentata* against *Pheretima posthuma*. Extracts were prepared using ethyl acetate, ethanol, and water at concentrations 25–100 mg/mL. Albendazole served as the standard drug. All extracts showed significant dose-dependent activity, with aqueous extracts displaying the highest potency, approaching the efficacy of albendazole.

Keywords*:Achyranthes aspera*, *Achyranthes bidentata*, Anthelmintic activity, Earthworms, Helminthiasis, Medicinal plants.

1. INTRODUCTION

Helminthiasis remains a major public health and veterinary concern, particularly within tropical and subtropical regions where sanitation infrastructure is limited. These parasitic infections lead to nutritional deficiencies, impaired cognitive development in children, reduced productivity in adults, and significant economic losses in animal husbandry. In livestock, helminth infestations negatively impact meat, milk, and wool production, making parasite control a priority for sustainable agriculture[1]. The reliance on synthetic anthelmintics such as albendazole, mebendazole, and ivermectin has undeniably improved disease management. However, excessive or improper usage has accelerated the emergence of drug-resistant helminth strains. Additionally, the high cost, limited distribution networks, and concerns about chemical residues in food animals highlight the need for safer, more affordable, and environmentally sustainable

alternatives[2]. Herbal medicine offers a promising avenue for developing such therapeutics. Plants contain a wide array of secondary metabolites capable of targeting multiple physiological pathways in parasites, reducing the risk of resistance development. Phytochemicals such as saponins disrupt parasite cell membranes, while tannins can interfere with energy metabolism and reproductive processes. With advances in chromatography, spectrometry, and molecular docking techniques, bioactive compounds can be more precisely identified, characterized, and optimized for medicinal use[3]. Belonging to the genus *Achyranthes*(family Amaranthaceae), including *Achyranthes aspera* and *Achyranthes bidentata*, have long been incorporated into traditional medicinal systems such as Ayurveda, Siddha, and Chinese herbal medicine. Ethnobotanical reports document their use for conditions ranging from digestive disorders to infectious diseases. Modern pharmacological studies further support their antimicrobial, anti-inflammatory, and antiparasitic properties. Extracts from various parts of *Achyranthes* plants leaves, roots, and seeds have exhibited significant in vitro and in vivo anthelmintic activity, particularly against nematodes[4]. The rich phytochemical profile of *Achyranthes* comprising alkaloids, flavonoids, sterols, glycosides, and notably saponins aligns with known antiparasitic mechanisms. Continued investigation into extraction methods, compound isolation, and mechanism-based assays is essential to validate therapeutic efficacy and safety. Furthermore, integrating traditional knowledge with modern pharmaceutical approaches may lead to novel plant-based formulations capable of addressing the global burden of helminthiasis, especially in resource-limited communities[5].

2. PLANT PROFILE

Figure:1 Plant Profile *(Achyranthus Aspera)*

Figure*:2 Achyranthus Bidentata*

2.1 Taxonomical Classification

Category	Classification
Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Caryophyllidae
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Achyranthes*
Species	*A. aspera*, *A. bidentata*

2.2. Bionomical Description

Achyranthes Aspera

A.aspera is an erect herb, ranging between 0.3 to 0.9 m height, stem stiff, branched stems are angular, ribbed and simple or branched from the base, often with tinged purple colour, and branches are quadrangular with thick leaves. Leaves are opposite, velvety, tomentose, obovate, margins wavy, surface covered with whitish hair. Petiole shows crescent shaped outline, having single layered epidermis with thick cuticle. Midrib shows a single layered epidermis, and on both surfaces, epidermis followed by 4-5 layered collenchyma on upper side and 2- 3 layered on lower side The flowers are bisexual, greenish-white and are arranged in a spike form. The bracts surround the flower in the fruiting stage and have sharp pointed tips making the head spiny to touch. Stamen 2-5, filament form, mono adelphous, alternating with quadrate. Styleslender, stigma small capitate and ovary is with pendulous ovule. Stem shows 6-10 ridges, which diminish downward to the base where it become almost cylindrical, epidermis single layered, covered by thick cuticle having uniseriate, 2-5 celled covering trichomes. Cortexis 6-10 layered, composed of parenchymatous cells[6].

2.3. Achyranthes Bidentata

Ox Knee is an erector straggling herb, 0.6-2m, much-branched. Stem and branches are indistinctly quadrangular or channeled, hair less to moderately (rarelymoredensely) hairy, the nodes frequently much shrunken when dry. Leaves are elliptic-oblong to broadly oval, rarely narrowly lance shaped, shortly or long-pointed, gradually or more abruptly narrowed below, 9-22 x 2.5-8.5 cm, usually thinly hairy, rarely densely apprised-hairy on the lower surface. Stalks of main stem leaves are 0.3-2 cm long, shortening above and below. Inflorescences at

first dense, finally lax and elongating to smooches 20cm but commonly about half this length, the inflorescence stalks 1-4cm. Bracts are narrow lance shaped, brownish-membranous, 3-5mm, hairless. Bracteoles are 3.5-5.5 mm. Tepals are 5, 4-7 mm, the outer longest, all narrowly lance shaped, very acute, with a distinct midri band 2 obscure or obvious lateral nerves, narrowly pale-margined. Capsuleis 2- 3 mm. Seed filling the capsule, cylindrical smooth recent discoveries[7].

3. PHYTOCHEMICAL STUDIES

3.1. Extractive Values

3.1.1. Determination of total ash

For determination of the total ash value, 2 gms of air dried drug powder was placed as a uniform layer in crucible silica. It was ignited on the burner and heated at 550±50°C in muffle furnace until it was white, indicating the absence of carbon. It was allowed to cool down in desiccators and weighed to determine the percentage of ash with reference to air-dried drug. Silica crucible was placed again in furnace for 30 minutes and cooled in desiccators and weighed. This process was repeated until the constant weight of crucible was obtained.

Total ash = weight of ash/weight of the crude drug taken x 100

3.1.2. Determination of solvent extractive values

5gm of the air dried, powdered macerated with 100 ml of solvent for 24 hours, shaken frequently and allowed to stand for 24 hrs. Thereafter, filtered, evaporated the filtrate to dried and weight was taken.

Determination of acid - insoluble ash

For determination of acid-insoluble ash, the ash was boiled with 25 ml of dilute HCl for 5 min and it was filtered through an ash-less Whatman filter paper and washed with hot distilled water until it became acid-free. Then, the paper was placed in silica crucible and burnt in a muffle furnace. When it became carbon-free, it was placed in desiccators to cool down, and weight was taken and calculated

Acid insoluble ash = weight of ash/weight of the crude drug taken x 10

3.1.3. Determination of Water-Soluble Ash Content

Total ash was boiled with water for 5 minutes and insoluble ash was collected in a sintered glass crucible washed ignited at a temperature not exceeding 450 ºC. Cool and weighed for the determination of water-soluble ash.

3.1.4. Determination of alcohol soluble extractive

Test sample was macerated with 100ml of alcohol in a closed flask for 24 hours, shaking frequently during 6 hrs and then it was allowed to stand for 18 hrs. Filter rapidly, taking precautions against loss of solvent. Evaporate 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dry 105 ºC to constant weight and weigh. Calculate the percentage of alcohol soluble extractive in comparison with air dried drug.

4. MATERIALS AND METHODS

Preparation of Plant Material: The dried plant (such as leaves or whole plant parts) is ground into a fine powder. The apparatus is assembled with a round-bottom flask, extractor body, and condenser. The powdered plant material is placed in the Soxhlet thimble, and an appropriate solvent (commonly petroleum ether, chloroform, ethyl acetate, methanol, or water) is added to the round-bottom flask. The solvent is heated to evaporate and then condenses in the condenser. The condensed solvent percolates through the sample, dissolving the extracts, which siphons back into the flask[8-11]. This cycle repeats continuously. The extraction (Figure 3) typically lasts for about 6 hours at controlled temperatures, often around 40 ˚C for methanolic extracts. Concentration of Extract: The extract obtained is concentrated by evaporating the solvent, often using a water bath or rotary evaporator, resulting in a semi-solid or dry extract stored appropriately.

Figure:3 (Soxhelt extraction of *Achyranthus Linn*)

The assay for anthelmintic activity was carried out as per the method of Ajaiyeoba *et al*., with insignificant modifications. The test was conducted by using the adult earthworm,

Pheretima posthuma, because of its anatomical and physiological similarity with the intestinal round-worm parasite of human beings. Earth worms have been used widely for the preliminary evaluation of anthelmintic activity *invitro* because of easy availability and ease of use. All the worms were washed with normal saline water to remove all fecal matters. The extracts are prepared with three different solvents for the two different species with the solvents aqueous, ethanol and ethylacetate[12-15]. The extract was prepared at the concentration of 100 mg/ml for four different concentrations six worms (Pheretima posthuma) of 8-10 cm were placed in petridish containing 30ml of above test extracts of the three different species of achyranthes. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed with fading away of their body colors.

Aqueous extract of *A.aspera* containing worms

Aqueous extract of *A.bidentata* containin gworms.

5. RESULTS AND DISCUSSION

5.1. Physiochemical Parameters

The analysis of physiochemical parameters determines the total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive is tabulated.

The observed results of physiochemical analysis were tabulated in the table 01 and 02.

Tab1e 01: Physiochemical properties of *Achyranthes aspera*

Particulars	Percentage
Total ash	9.59%
Acid insoluble ash	2.27%
Water soluble extractive	20.91%
Alcohol soluble extractive	22.33%
Soluble solvents	Water, Ethanol, Ethyl acetate

Table 02: Physiochemical properties of *Achyranthus bidentata*

Particulars	Percentage
Total ash	8.50%
Acid insoluble ash	2.12%
Water soluble extractive	20.46%
Alcohol soluble extractive	21.87%
Soluble solvents	Water, Ethanol, Ethyl acetate

5.2. PRELIMINARYPHYTOCHEMICALTESTS

Preliminary phytochemicals test reveals the presence of alkaloids, flavonoids, tannins, saponins. The observed results were tabulated in table 3,4,5,6 and 7. These phytochemicals are responsible for potential anthelmintic property *of A. aspera* and *A. bidentata*

Table 03: Preliminary Phytochemical Tests

Phytochemicals	Result	
	A.aspera	***A.bidetata***
Alkaloids	present	Present
Flavonoids	present	present
Steroids	absent	absent
Triterpenoids	absent	absent
Tannins	present	Present
Saponins	present	Present
Phenol	absent	absent
Carbohydrate	present	Present

Table 04: Anthelmintic activity of *Achyranthes aspera* extracts earthworms

Drug	Concentration	Time taken for Paralysis (min)	Time taken for Death (min)
Ethylacetate	25	44.80	58.12
	50	33.30	43.28
	75	31.15	38.10
	100	34.50	38.55
Ethanol	25	59.50	72.30
	50	51.10	63.20
	75	40.20	52.55
	100	28.55	37.55
Aqueous	25	35.45	50.10
	50	26.54	38.48
	75	18.10	27.16
	100	15.40	21.12

Table 05: Anthelmintic activity of *Achyranthes bidentata* extracts on earthworms

Drug	Concentration	Time taken for Paralysis (min)	Time taken for death(min)
Ethylacetate	25	44.20	59.12
	50	34.30	44.28
	75	31.45	38.10
	100	35.20	39.25
Ethanol	25	60.30	73.10
	50	51.40	63.50
	75	40.50	53.15
	100	29.05	36.25
Aqueous	25	36.25	51.10
	50	26.58	38.57
	75	18.30	27.36
	100	15.50	21.20

Table 06: Anthelmintic activity of *Achyranthes bidentata* extracts on earthworms

Drug	Concentration	Time taken for paralysis(min)	Time taken for death(min)
Ethylacetate	25	44.20	59.12
	50	34.30	44.28
	75	31.45	38.10
	100	35.20	39.25
Ethanol	25	60.30	73.10
	50	51.40	63.50
	75	40.50	53.15
	100	29.05	36.25
Aqueous	25	36.25	51.10
	50	26.58	38.57
	75	18.30	27.36

	100	15.50	21.20

Table 07: Anthelmintic activity of standard drug Albendazole on earthworm

Drug	Concentration	Time taken for paralysis (min)	Time taken for death(min)
Albendazole	25	24.18	45.17
	50	21.34	46.55
	75	13.30	33.55
	100	11.10	26.54

From the results we can interpret that the motility of the worms is lost on exposure to the aqueous, ethyl acetate, ethanol extracts of *achyranthes aspera* and *achyranthes bidentata*. All three extracts have shown progressive dose dependent paralysis ranging from the loss of motility to loss of response to the external stimuli at the tested concentration of about 25, 50, 75 and 100mg/ml respectively. Among the three extracts of the *A.aspera* species, the aqueous extract proves to be more efficient and has shown greater activity when compared with the other extracts (ethanol, ethyl acetate). Aqueous extract could produce paralysis within 35.45, 26.54, 18.10 and 15.40 min at 25, 50, 75, and 100 mg/ml respectively. Ethyl acetate extract has produced paralysis within 44.80, 33.30, 31.15 and 34.50 min at 25, 50, 75 and 100 mg/ml respectively. The ethanol extract has shown less efficiency in the activity when compared with other two extracts. It produces paralysis at 59.50, 51.10, 40.20 and 28.55 min at 25, 50, 75 and 100mg/ml respectively. Among the three extracts of the *A. bidentata* species the aqueous extract proves to be more efficient and has shown greater activity when compared with the other drug extracts (ethanol, ethyl acetate). Aqueous extract could produce paralysis within 35.45, 26.54, 18.10 and 15.40min at 25, 50, 75 and 100mg/ml respectively. Ethyl acetate extract has produced paralysis within 44.80, 33.30, 31.15 and 34.50 min at 25, 50, 75 and 100 mg/ml respectively. The ethanol extract has shown less efficiency in the activity when compared with other two extracts. It produces paralysis at 59.50, 51.10, 40.20 and 28.55min at 25, 50, 75 and100mg/ml respectively. The standard drug albendazole has caused paralysis within 24.18, 21.34, 13.30 and 11.10 at 25, 50, 75, 100 mg/ml respectively. Paralysis eventually progressed to death of the earthworms. Mortality was produced at 50.10, 38.48, 27.16 and 21.12min for aqueous extract of *A.aspera* at

25, 50, 75 and 100mg/ml respectively. Ethyl acetate extract produces death at 58.12, 43.28, 38.10 and 38.55 min at 25, 50, 75, 100mg/ml respectively. When compared to the other two extracts the ethanol extract takes longer time for death of the worms. The time taken by the ethanol extract of *A. aspera* to causes death of the earthworms at 25, 50, 75 and 100mg/ml at 72.30, 63.20, 52.55 and 37.55 minute. The standard drug albendazole caused death at 45.17, 46.55, 33.55 and 26.54min at 25, 50, 75 and 100mg/ml respectively. From the above observations, it is evident that all the three extracts of both the plant extracts show anthelmintic 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 found to be close to that of standard drug. The aqueous extracts of the two species show similar result[16-17]. The times taken for the death of worms on both extracts were almost equal to each other thus providing the fact that the efficiency of anthelmintic activity was similar. The presence of phytochemicals like alkaloids, flavonoids, phenols, tannins, glycosides, carbohydrates in various parts of *A.aspera* and *A. bidentata* have been reported by many researchers. The anthelmintic activity of the selected medicinal plant might be due tothe presence of these phytochemicals.

6. CONCLUSION

Both *Achyranthes aspera* and *Achyranthes bidentata* possess strong anthelmintic activity, validating their traditional use. Among all preparations, aqueous extract at 100 mg/mL exhibited the highest potency, closely approaching standard albendazole. These plants can serve as promising herbal anthelmintic agents and may offer an alternative in combating drug-resistant helminths.

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