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RESEARCH ARTICLE

INVITRO ANTHELMINTIC ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF *ACHYRANTHES ASPERA*

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ABSTRACT

The present work deals with the anthelmintic and preliminary phytochemical analysis on leaves of *Achyranthes aspera* Amaranthaceae. The anthelmintic activity performed on ethanolic extract (10,15 and 25mg/ml) and aqueous extract(25,50 and 100mg/ml) of leaves of *Achyranthes aspera* by using *Pheretima posthuma* as test worms. The time of paralysis and time of death were studied and the activity was compared with Piperazine citrate as reference standard and distilled water as control. Dose dependent activity was observed in both the extracts. Ethanolic extract was found to be more potent than aqueous extract. Preliminary phytochemical analysis shows the presence of glycosides, terpenoids, alkaloids, tannins, coumarins, quinines, phenolic compounds. The results indicate that the leaf extracts of *Achyranthes aspera* recommend to use in preparation of herbal drugs.

Keywords: *Achyranthes aspera* Linn. , *Pheretima posthuma*, Anthelmintic activity, Ethanolic extract, aqueous extract, Piperazine citrate, Distilled water, Phytochemical analysis.

INTRODUCTION

Since the time immemorial, our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully including antibacterial, anthelmintic and anti-inflammatory etc. Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. 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 undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting population in endemic areas[5]. Most of the existing anthelminthics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea. Since ancient times herbal drugs are used for the treatment of parasitic diseases in human without any side effects. To eradicate the side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient people's used[1]. Hence there is an increasing demand towards natural anthelmintics.

Anthelmintics are drugs that act locally to expel worms from the gastro-intestinal tract or systemically to eradicate adult helminths or developmental stages that invade organs and tissues. These medicines are used in human and other animal populations to destroy parasites that live in the body. According to World Health Organization statistics, more than two billion people harbour parasitic worm infections. In areas of high prevalence, simultaneous infection with more than one type of helminths is common. One of the problems with anthelmintics is that many of them have been used for a long time and over time parasites have developed drug resistance[6].

Achyranthes aspera Linn. Is known as uttareni. It belongs to family Amaranthaceae. *Achyranthes aspera* L. (Latjeera) is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour, branches terete or absolutely quadrangular, striate, pubescent , leaves thick , 3.8 - 6.3 × 22.5 - 4.5 cm , ovate – elliptic or obovate – rounded , finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long , flowers greenish white, numerous in axillary or

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terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown[2].

In the present communication the preliminary phytochemical analysis of different extracts of this plant was found to have various active chemical moieties.

Uses: *Achyranthes aspera* Linn found to have Spermicidal Activity, Antiparasitic Activity, Hypoglycemic Activity, Cancer Chemo preventive Activity, Hepatoprotective Activity, Analgesic and antipyretic activity, Anti-inflammatory and anti-arthritic activity, Antimicrobial Activity, Anti-oxidant Activity, Nephroprotective Activity, Anti-depressant Activity, Diuretic Activity, Bronchoprotective Activity, Cardiovascular Activity, Anti-allergic Activity, Wound Healing Activity, Immunomodulatory Activity, Hypolipidemic Activity[2].

MATERIALS AND METHODS

Drugs and chemicals: Piperazine citrate and Distilled water

Reagents and Solvents: Mayer's reagent, Dragendorff's reagent, Fehling solutions A and B, dilute HCl, Barfoed's reagent, Benedict's reagent,NH_3, Millon's reagent, ethanol (95%),$CHCl_3$, 2%$CuSO_4$, KOH pellets, Ninhydrin reagent, acetic anhydride, conc. H_2SO_4, Lead acetate**,** $FeCl_3$, NH_4OH, NaOH, Glacial acetic acid, $NaHCO_3$, phenolphthalein, Ethanol AR.99.9% (Changshu Yangyuan Chemicals).

Plant material: The plant *Achyranthes aspera* was collected from Mindivanipalem village, Vizag district, Andhra Pradesh during the month of November 2012. The plant material was identified and authentified by Dr.M.V.R.K. Narasimhacharyulu, MSc, M. Phil, Ph.D, Principal of Sri Y.N.College of Botany, Narasapuram, West Godavari district, A.P. The collected leaves were shade dried under normal environmental conditions, powdered, stored at 4-6^{0}C in refrigerator, in a closed container for further use.

Preparation of Plant Extracts

Aqueous Extract: The leaves of the plant was dried in shade and made into fine powder using a laboratory mill. The dry powder (45g) is extracted with distilled water (250ml) using maceration process for about 72 hours at room temperature. The water soluble materials were filtered by using muslin cloth. The excess solvent in the extract were removed by evaporation on water bath to get thick paste. The extract was collected in a petridish and stored at 2-6 ^{0}C.

Ethanolic Extract: The leaves of the plant was dried in shade and made into fine powder using a laboratory mill. The dry powder (48g) is extracted with ethanol (250ml) using maceration process for about 72 hours at room temperature. The ethanol soluble materials were filtered by Buchner funnel. The excess solvent in the extract was removed by distillation and concentrated on water bath to get thick paste and collected in a petridish and stored at 2-6 ^{0}C. The yield value and other physical properties were observed.

Animals: Indian earthworm *Pheretima posthuma* were collected from the water logged areas of soil in chintalagraharam village, Visakhapatnam, Andhra Pradesh. The worms were transferred into a glass bottle with some quantity of the soil from which they were taken. They were washed with water to remove dirt. The length of *Pheretima posthuma* was 6-8 cm and width is 0.1-0.2 cm respectively. They were washed with water to remove dirt. *Pheretima posthuma* has been used as test worm in most of the anthelmintic activities, as it shows anatomical and physiological resemblance with the intestinal round worm parasite of humanbeings[3,4,5].

Anthelmintic activity: The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al* with minor modifications [6,7] .The assay was performed *invitro* using adult earthworm (*Pheretima posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of antihelmintic activity of ethanolic extract was prepared at the concentrations 10, 15, and 25 mg/ml in 25ml of distilled water. Similarly aqueous extract was prepared at 25, 50, and 100 mg/ml in 25ml of distilled water and six worms i.e. *Pheretima posthuma,* of approximately equal size were placed in each 9.8 cm petridish containing 25 ml of above test solution of extracts. Piperazine citrate (10mg/ml) was used as reference standard and distilled water as control [4,5,6] . All the test solution and standard drug solution were prepared freshly before starting the experiment.

Observations were made for the time taken for paralysis when no movement could be observed and when the worms do not retrieve 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 (50 ^{0}C). All the results were shown in Table.3 and expressed as a min ±sec of six worms in each group.

Preliminary phytochemical analysis: The property of selective reactivity of phytochemical present in ethanol and aqueous extracts forms the basis of chemical tests for identification of different constituents. The preliminary analysis of *Achyranthes aspera* leaf was performed initially to identify various chemical compounds present and to asses physicochemical properties (Table 4).

RESULTS AND DISCUSSION

From the results shown in table no.4, Preliminary phytochemical screening of Ethanolic extract revealed the presence of Glycosides, Phenols, Coumarins, Tannins, Flavanoids, steroids and Terpenoids. While aqueous extract showed the presence of alkaloids, phenols, Coumarins and quinones. From table no.3 the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis[11].

The Ethanolic leaf extract of *Achyranthes aspera* demonstrated paralysis as well as death of worms in almost equal time as compared to piperazine citrate especially at higher concentration of 25mg/ml. While aqueous extract also shown significant activity. Phytochemical analysis of *Achyranthes aspera* revealed presence of phenols as one of the common chemical constituent in both the crude extracts. Polyphenolic compounds show anthelmintic activity. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that phenolic content in the extracts of *Achyranthes aspera* produced similar effects[12].

CONCLUSION

In conclusion, it was observed that the Aqueous and Ethanolic extracts obtained from the leaves of *Achyranthes aspera* Linn. possess good anthelmintic activity, but the ethanolic extract had shown better anthelmintic activity when compared to aqueous extract and standard drug Piperazine citrate. In order to confirm the above results, the *in vivo* studies have to be conducted.

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Table 1: Colour, Odour, consistency and % yield of *Achyranthes aspera*

PHYSICAL CHARACTERCS	COLOR	ODOUR	CONSISTENCY	% YIELD
Aqueous extract	Greenish Brown	characteristic	Viscous pasty mass	12.9%
Ethanolic extract	Greenish Brown	characteristic	Viscous	4.84%

Table 2: A guide to ascertain paralysis and death of experimental worms[6]

Parameter	Description
Paralysis	Marked decrease in vigorous wriggling movement of the worm indicates paralysis. If the worm revived in saline solution.
Death	Evoked pin prick response: Slow movement of the worm after being pricked with pin indicates paralysis. No movement indicates death. Confirmed by dipping the worm in warm water at 50 ^{0}C and shaken vigorously. No response indicates death.

Table 3: Anthelmintic activity of *Achyranthes aspera* leaves

Groups	Concentration (mg/ml)	*Pheretima posthuma* (Earthworm)	
		Time taken for Paralysis (P) (min ±sec)	**Time taken for death (D)** (min ±sec)
Control (Water Only)	–	–	–
Piperazine citrate(Standard)	10	24±36	63±54
Ethanolic extract	10	34±58	120±16
	15	30±33	101±32
	25	24±43	64±23
Aqueous extract	25	28±34	110±46
	50	15±56	50±16
	100	8±32	24±28

Table 4: Qualitative phytochemical analysis of *Achyranthes aspera* extracts

Phytochemical test	Aqueous extract	Ethanolic extract
Alkaloids		
Dragendroff's test	+	–
Mayer's test	–	–
Carbohydrates		
Fehling's test	–	–
Barfoed's test	–	–
Benedict's test	–	–
Glycosides		
Legal test	–	–

Cardiac glycoside test	_	+
Fixed oils & Fats		
Stain test	_	_
Saponification test	_	_
Phenolic compounds		
Lead acetate test	+	+
Coumarins	+	+
Tannins	_	+
Flavanoids	_	_
Steroids(phytosterols)	_	+
Detection of acids	_	_
Quinones	+	_
Saponins	_	_
Gum & mucilage	_	_
Anthraquinones	_	_
Proteins		
Millions test	_	_
Biuret test	_	_
Amino acids		
Ninhydrin test	_	_
Terpenoids	_	+

Note: (+) positive test, (-) negative test.