

ANTHELMINTIC ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF *ANANAS COMOSUS* LINN.

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ABSTRACT

The present study aimed at the *in-vitro* evaluation of anthelmintic activity of aqueous and methanolic extracts of leaves of *Ananas comosus* (Bromeliaceae) using four different concentrations (25, 50, 75 and 100 mg/ml) respectively. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. At the concentration of 100 mg/ml both the methanolic and the aqueous extracts exhibited very significant activities as compared to the standard drug piperazine citrate (10 mg/ml) and Albendazole (20 mg/ml). The time of paralysis and death was recorded as 8.55 ± 0.10 (P) and 32.25 ± 0.25 (P) in case of aqueous extract, on the other hand it was recorded as 8.50 ± 0.37 (P) and 32.10 ± 0.53 (P) in case of methanolic extracts. In conclusion, the use of leaves of *Ananas comosus* as an anthelmintic have been confirmed and further studies are suggested to isolate the active principles responsible for the activity. Both the extracts showed significant anthelmintic activity.

Keywords: Anthelmintic, *Pheretima posthuma*, *Ananas comosus*, Piperazine citrate, Albendazole

INTRODUCTION

Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries due to poor management practices¹. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases². Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human³.

Pineapple (*Ananas comosus*) native to Central and South America, is grown in several tropical and subtropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines and Thailand. It has been used as a medicinal plant in several native cultures and bromelain has been chemically known since 1876⁴.

Botanicals such as *A. comosus* (Pineapple) and their extracts (bromelain) have been used clinically as anti-inflammatory agents in rheumatoid arthritis, soft tissue injuries, colonic inflammation, chronic pain and asthma^{5,6,7}. Bromelain have been reported to have anti-diarrhoeal activity⁸, is also useful in the prevention and treatment of thrombosis and thrombophlebitis, break down cholesterol plaques and exerts a potent fibrinolytic activity⁶. Literature review indicates that anthelmintic activity of this species has not been clinically evaluated so far. The present paper reports the anthelmintic activity of methanolic and aqueous extracts of *A. comosus* against Earthworms.

MATERIALS AND METHODS

Plant material

As northeastern states are the major producers of pineapple in India, The leaves of *Ananas comosus* (Bromeliaceae) were procured from Sibsagar District (Assam) in September 2010. The plant and plant material were identified and authenticated in Department of Life science, Dibrugarh University, Dibrugarh, Assam and Voucher herbarium specimen was deposited in the Department of Pharmacognosy of ROFEL, Shri G.M.B college of Pharmacy for future reference bearing a No-RPC/RS/001. The plant material was dried in sunlight, pulverized, passed through sieve no. 20 and stored in air tight container and used for further extraction.

Preparation of extract

Fresh leaves of *A. Comosus* were collected and air dried in shade at room temperature for 15 days. The powdered plant material was subjected to successive solvent extraction with petroleum ether (60-80%) chloroform, methanol in Soxhlet extractor and cold maceration

of the successive marc in water. The extracts were dried at low temperature under reduced pressure. The preliminary phytochemical investigation was carried out of methanol and aqueous extracts.

Worm collection and authentication

Indian adult earthworms (*Pheretima posthuma*) were collected from moist soil and water logged areas at Vapi, Namdha Road and were identified at the Department of Pharmacology, ROFEL, Shri G.M.B college of Pharmacy (Faculty of Pharmacy) Vapi. Then all collected worms were washed with normal saline to remove all the faecal matter and used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol.

Sample preparation

Test sample for *in vitro* study were prepared by dissolving and suspending 2.5 gm of methanol extract in minimum amount of DMF and the volume was adjusted to 25 ml with normal saline to obtain a stock solution of concentration of 100 mg/ml. From this stock solution different dilutions were made to get concentration range of 25, 50, 75 and 100 mg/ml.

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline), Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Chemicals: Methanol A.R (PCL, Pune), DMF (PCL, Pune), Saline water (Claris Lifesciences Ltd., Ahmedabad).

Anthelmintic activity

The anthelmintic activity was performed according to the method of Ghosh et al.⁹ on adult Indian earthworm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings^{10,11}. *Pheretima posthuma* worms are easily available and used as suitable model for screening anthelmintic drugs¹². In the 50 ml of formulations containing four different concentration of methanolic extract and aqueous extract (25, 50, 75 and 100 mg/ml in normal saline) and the standards Piperazine citrate (10 mg/ml) and Albendazole (20 mg/ml) were prepared and approximately equal sized six earthworms were released in each group. Observations were made for the time taken to paralyse or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. Piperazine citrate (10 mg/ml) and Albendazole (20 mg/ml) were used as reference standards and normal saline water as control.

RESULTS

Preliminary phytochemical screening of plant of *A.comosus* showed the presence of alkaloids, terpenoids, flavonoids, steroids, tannins and resins. As shown in table-I, methanolic and aqueous extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The alcoholic extract of *A.comosus* linn. caused paralysis (P) of 8.50 min. and time of death (D) of 32.1 min. while aqueous revealed paralysis of 8.55 and death of 32.25 min. respectively against the earthworm *Pheretima posthuma*. The reference drug Piperazine citrate showed the same at 22.77 and 61.01 minutes and Albendazole showed the same at 31.86 and 42.50 minutes respectively.

Table 1: Anthelmintic activity of methanolic and aqueous extracts of *A.comosus*

Test substance	Concentration (mg/ml)	Time taken for paralysis (min.)	Time taken for Death (min.)
Vehicle	-----	No paralysis (upto 72 min)	No death (upto 72 min)
Piperazine citrate (Std.)	10	22.77±0.27**	61.01±0.42**
Albendazole (Std.)	20	31.86±0.22**	42.50±0.26**
Methanolic extract	25	36.35±0.23**	72.10±0.33**
	50	23.59±0.29**	64.1±0.24**
	75	18.09±0.07**	43.91±0.26**
	100	8.50±0.37**	32.10±0.53**
Aqueous extract	25	34.94±0.93**	69.05±0.28**
	50	28.43±0.18*	48.83±0.29**
	75	19.29±0.20**	51.6±0.21**
	100	8.55±0.10**	32.25±0.25**

Each value represents mean ± SEM (N=6). *P<0.05, **P<0.01. This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.

DISCUSSION

Albendazole by increasing chloride ion conductance of worms muscle membrane produced hyperpolarization and reduced excitability that which led to muscle relaxation and flaccid paralysis¹³. Piperazine citrate acts by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds. It is possible that tannins contained in the extracts of *A.comosus* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. The exact

mechanism of the anthelmintic activity of *A.comosus* cannot be explained on the basis of our present results. From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

CONCLUSION

From the above results, it is concluded that *A.comosus* used traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent responsible for anthelmintic activity.

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