



IN-VITRO ANTHELMINTIC ACTIVITY OF BENINCASA HISPIDA (PETHA) THUNB LEAVES

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ABSTRACT

The present study was undertaken to evaluate anthelmintic activity of crude aqueous, Petroleum ether, chloroform and Methanol extract of *Benincasa hispida* (Petha) leaves using *Pheretima posthuma* as test worms. Single concentration (5 %) of extracts was tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was included as standard reference and distilled water as control. The results of present study indicated that *Benincasa hispida* (Thunb) fresh leaves extracts were exhibited anthelmintic activity significantly (***) $p < 0.001$ when compared with standard (Piperazine citrate) group. Chloroform extract (30.6 ± 1.364 minutes & 56.8 ± 1.497 minutes) and petroleum ether extract (55.8 ± 2.518 minutes & 80.4 ± 2.909 minutes) showed shortest time of paralysis (P) and death (D) with 50 mg/ml concentration respectively among all extracts. Whereas, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. Further studies are in process to isolate the active principle/s responsible for the activity.

KEY WORDS

Anthelmintic, *Benincasa hispida*, *Pheretima posthuma*

INTRODUCTION

The fruit of *Benincasa hispida* (Thunb) Cogn, commonly called as ash guard, belonging to cucurbitaceous is employed as a main ingredient in kusmanda lehyam, in Ayurvedic system of medicine. The lehyam is used as rejuvenate agent and also numerous nervous disorders. Many empirical applications have been used in India centuries for various ailments such as GIT problems such as dyspepsia, burning sensation, heart disease, vermifuge, diabetes, and urinary disease [1, 2]. Though some scientific studies have been carried out reveal its anti-

inflammatory activity [3], diuretic activity [4], Hypoglycemic [5], Anti Alzheimer's [6], Antidiarrheal [7], antioxidant [8], Antiulcer [8-9], anti-obesity [10], antihistaminic [11] and anti cancer [12]. The major constituents of this fruits are triterpenoids, flavanoids, glycosides, saccharides, carotenes, vitamins, β sitosterin, and uronic acid [13- 15]. However there is no report on anthelmintic activity of this plant. In the light of the above information the present investigation was undertaken to evaluate the anthelmintic potential of *Benincasa hispida* leaves extract and is being reported here. Keeping these views in mind, present



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study was planned to evaluate anthelmintic activity of leaves extracts. department of Biotechnology, Government of India, Takyelpat, Imphal, Manipur-795001, India.

MATERIALS AND METHODS

Plant Material

Benincasa hispida (Thunb) Cogn fresh leaves were collected from forest of kanchanpur, Tripura North, India in January 2006 and identified by Professor K. Prabhu (Botanist and Professor in Department of Pharmacognocny, S.C.S. College of Pharmacy, Harapanahalli, Karnataka) where a voucher specimen (PP/007/2006) was deposited for reference to Department of Pharmacognosy, S.C.S. college of pharmacy, Harapanahalli, Karnataka.

Preparation of Extract

Shade-dried leaves powder was extracted with petroleum ether, Chloroform, methanol (90%) and distilled water by soxhletion. The extract was concentrated by rotary vacuum evaporator. The dried extract was stored in air tight container in refrigerator below 10°C. The extract was suspended in distilled water for experiments.

Worms Collection and Authentication

Indian earthworm *pheretima posthuma* (Annelida) were collected from the water logged areas of solid and identified at the Microbial resources Division, Institute of Bioresources and sustainable Development,

Preparation of test sample

Samples for in-vitro study were prepared by dissolved 5 gm extract in 100 ml purified water to make 5 % solution of test extracts. 10 ml of same solution was taken in each respective petridishes.

Anthelmintic assay

For the Anthelmintic activity of leaves extracts of *Benincasa hispida* (Thunb) Cogn, Indian adult earthworms (*pheretima posthuma*) of 3-5 cm in length and 0.1 – 0.2 cm in width were used. The earthworms were divided into six groups containing five earthworms in each group. All the extracts were freshly prepared in 5% concentration before starting the experiments. Different extracts were poured in different petridishes. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows: Distilled water (10 ml), Piperazine citrate (10 mg/ml), petroleum ether (50 mg/ml), Chloroform extract (50 mg/ml), Methanol extract (50 mg/ml), and aqueous extract (50 mg/ml). Observation were made for the time taken to paralysis (paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously) and Death (Death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate was used as reference standard while distilled water as control. [16-17]



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Table 1.
In-vitro Anthelmintic activity of Benincasa hispida (Petha) Thunb leaves extracts.

S.No	Treatment	Concentration (mg / ml)	Time taken for paralysis and death of worms in minutes	
			Paralysis	Death
1	Control (dist water)	10	---	---
2	Piperazinecitrate (standard)	10	007.6 ± 0.927	015.8 ± 1.158
3	Petroleum ether extract	50	055.8 ± 2.518	080.4 ± 2.909
4	Chloroform extract	50	030.6 ± 1.364	056.8 ± 1.497
5	Methanol extract	50	124.6 ± 3.059	177.4 ± 3.501
6	Aqueous extract	50	111.8 ± 2.557	156.6 ± 5.636

--- means no paralysis or death, Values are mean ± SEM (n=5)

*** p<0.001 vs. standard group, one way ANOVA test.

RESULTS AND DISCUSSION

The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [18-20]. *Posthuma* worms are easily available and used as a suitable model for screening of Anthelmintic drug was advocate earlier [21-26].

In present study *Benincasa hispida* (Thunb) Cogn fresh leaves extracts were exhibited anthelmintic activity significantly (**p<0.001) when compared with standard group. Chloroform extract (30.6 ± 1.364 minute & 56.8 ± 1.497 minute) and petroleum ether extract (55.8 ± 2.518 minute & 80.4 ± 2.909 minute) showed shortest time of paralysis (P) and death (D) with 50 mg/ml concentration respectively among all extracts. Whereas, in control group, worms were observed for 24 hours and no paralysis or death was found during that period.

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane

produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [27].

The leave extract of *Benincasa hispida* not only demonstrated paralysis, but also causes death of worms especially at higher concentration of 50 mg/ml, in shorter time as compare to reference drug piperazine citrate.

CONCLUSION

On the basis of these investigations, we may partially conclude that *Benincasa hispida* could be a potent anthelmintic agent for next generation. Further studies are require on phytochemical profiling as well as isolation and identification of bioactive component responsible for anthelmintic activity.

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