



EVALUATION OF ANTHELMINTIC ACTIVITY IN THE BARK OF

*Homalium zeylanicum*K. Gnananath^{*1}, G. Pavan Kumar¹, K. Ramakanth Reddy¹, B. Naveen Kumar¹, R. Vinod Kumar²¹Dept. of Pharmacognosy, Netaji Institute of pharmaceutical sciences, Toopranpet, Nalgonda, Andhra Pradesh, India²Dept. of Pharmacognosy, Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Andhra Pradesh, India

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*Email: k.gnananath@gmail.com

ABSTRACT

The main aim of the study was to investigate Phytoconstituents and antihelmintic activity in the bark extract of *Homalium zeylanicum*. All phytochemical screening methods were done by using standards methods, where as antihelmintic method was done at three different concentration on *Pheretima posthuma*, the parameters like the time of paralysis and the time of death were determined. Both the extracts showed significant activity in the dose dependent manner.

Keywords: Phytochemical screening, Anthelmintic activity, *Homalium zeylanicum*, *Pheretima posthuma*.

INTRODUCTION

Helminthiasis is a macroparasitic disease which is common in both humans and animals in which a part of the body is infested¹. This infection is highly prevalent particularly in third world countries like India due to poor management practices. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver or other organs and cause serious damage². Though there are many antihelmintic drugs available in the market, there is rapid increase in the research and establishment of a new antihelmintic drug because of the development of resistance towards the drugs³. Hence the present focus is on discovery of new antihelmintic drug from the plants.

Homalium zeylanicum (Flacourtiaceae) is commonly known as "Liyan or Mukki". The bark and leaf of the plant is having many traditional uses in diabetes, rheumatism and wound healing activities⁴. Interesting note of this plant is that it is used since ancient in black remedies, the twigs of the plant is used in removal of evil spirits hence local name mantralamukhi. In the present study was investigated for the presence of anthelmintic activity.

MATERIALS AND METHODS**Collection of plant material:**

Homalium zeylanicum Plant bark was collected in and around Tirumala hills, Andhra Pradesh botanically identified and authenticated and a voucher specimen (KG-GP/2012) was deposited in Netaji institute of Pharmaceutical science, Toopranpet, Nalgonda, India for future reference. Bark was mechanically ground and obtained bark powder was used for further studies

Preparation of extract:

For the present study, the extracts were prepared by continuous hot percolation method using Soxhlet apparatus. 250 gm of bark powder was passed through sieve no. 60 and packed in Soxhlet apparatus and extracted using ethyl acetate and methanol as solvents. The filtrate was concentrated in rotary evaporator and the extracts were calculated for their yield and stored in desiccators. The extracts were designated as EAHM for Ethyl acetate and ALHM for methanol extract respectively. And these two extracts were subjected to

preliminary phytochemical screening using standard procedures

Phytochemical tests:

All phytochemical tests were done by using standard protocols^{5,6}.

Total Flavonoid content

Aluminum chloride colorimetric method⁷ was used for flavonoids determination. One milliliter (1 mL) of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with ultraviolet (UV) visible spectrophotometer. The content was determined from extrapolation of calibration curve which was prepared by preparing quercetin solution (2-10 µg/mL) in methanol. The concentration of flavonoid was expressed in terms of mg/mL.

Total phenolic content

The amount of total phenolic content of the extracts was determined by Folin-Ciocalteu reagent as oxidizing agent, gallic acid as standard⁸. Exactly 0.5 mL of the extract was transferred to a 100 mL erlenmeyer flask and the final volume was adjusted to 46 mL by addition of distilled water. 1 mL of Folin-Ciocalteu reagent was added and incubated at room temperature for 3 min. 3 mL of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as the standard (20-100 µg/mL) for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

Experimental Model

Adult earthworm *Phertima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil, obtained from agricultural fields nearby, Toopranpet of Nalgonda district, A.P.-India. Four test groups were taken each containing six earth worms of approximately equal size (8±1 cm). Albendazole was taken as standard drug (20mg/ml) was prepared in normal saline containing 5% DMF. The methanolic⁹⁻¹¹. *Homalium zeylanicum* extract of different concentrations were prepared by dissolving in minimum

quantity of DMF and making up to the final volume with normal saline to obtain 10mg/ml, 25mg/ml, and 50mg/ml concentrations. One of the groups is taken as control group which was treated with normal saline containing 5% DMF. Paralysis onset time and death time of individual worms were noted. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of color of worm.

RESULTS AND DISCUSSIONS

TABLE -1 PERCENTAGE YIELD OF DIFFERENT EXTRACTS OF *HOMALIUM ZEYLANICUM*

Extracts	Percentage of yield
EAHZ	4.5% w/w
ALHZ	3.6% w/w

The percentage yield was found to be 4.5 % w/w for ethyl acetate and 3.6 % w/w for methanol.

TABLE - 2 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACTS OF *HOMALIUM ZEYLANICUM*

Tests	EAHM	ALHM
Alkaloids	+	+
Glycosides	-	+
Tannins	+	+
Flavonoids	+	+
Carbohydrates	+	+

“+” indicates presence “-” indicates absence

By the above table it is clear that the *H. zeylanicum* bark contains all the phytoconstituents especially it showed the rich presence of tannins and flavonoid

Total flavonoid content

Total flavonoid content of EAHM, ALHM (1mg) equivalent to 3.76 µg, 3.56 µg respectively of quercetin was detected.

Total phenolic content

In the present study total phenolics content of ALEH, AQEH (1mg) equivalent to 67.5 µg and 47.54 µg respectively of gallic acid was detected.

TABLE - 3 ANTHELMINTIC ACTIVITY OF *HOMALIUM ZEYLANICUM*

TREATMENT	TIME TAKEN BY EARTHWORMS FOR	
	PARALYSIS (min)	DEATH (min)
CONTROL	—	—
AIBENDAZOLE	25.6±0.75	65±0.65
EAHM		
10 mg/ml	33±0.45	90±0.54
25 mg/ml	29±0.63	76±0.65
50 mg/ml	18±0.15	55±0.56
ALHM		
10 mg/ml	30.16±0.33	75.17±0.54
25 mg/ml	25±0.67	60.16±0.68
50 mg/ml	15±0.77	40.16±0.45

Preliminary phytochemical screening has shown the presence of alkaloids, tannins, flavonoids, triterpenes and

carbohydrates the crude extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The crude extracts of methanol showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results at different concentrations. Where as methanolic extract at 50 mg/ml showed better activity 40.16 min (death time) than standard albendazole

CONCLUSION

Thus the methanolic bark extract showed significant activity than that of standard drug albendazole. This might due to the presence of secondary metabolite tannins which are responsible for the activity.

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