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Evaluation of Anthelmintic activity of *Uncaria gambier R*oxb. against *Pheretima posthuma.*

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Abstract

The present study was designed to evaluate the anthelmintic potential of leaves & shoots extract of *Uncaria gambier* Roxb. The alcoholic extract of *Uncaria gambier* Roxb. & its ethyl acetate fraction at different concentrations (25, 50, 75, 100 mg/ml) were tested on Indian adult earthworms (*Pheretima posthuma)* by in vitro standard procedure. Time of paralysis and time of death of the worms were considered as the parameters to assess the anthelmintic action. Albendazole and 2% w/v gum acacia in distilled water were used as standard and control respectively. The ethyl acetate fraction of alcoholic extract exhibited potent anthelmintic activity compared to alcoholic extract as evidenced by significant decrease in time of paralysis & death. The observed activity could be due to the presence to phenolic compounds, particularly flavonoids in the test extract. These in vitro studies indicated that the *Uncaria gambier* Roxb. is a significant source of natural anthelmintic, which might be helpful in preventing the progress of various parasitic disorders.

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Uncaria gambier, Alcoholic extract, Ethyl acetate fraction, Paralysis, Death, Anthelmintic activity.

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Introduction

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 [1]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas[2]. Ideally an anthelmintic agent should have an broad spectrum of action, high percentage of cure with a single therapeutic dose, free from toxicity to the host & should be cost effective. None of the synthetic drugs available meets these requirements. Even most common drug like piperazine salts have been shown to have side effects like nausea, intestinal disturbances & giddiness[3]. Resistant of parasites to existing drugs & their high cost warrants the search for newer anthelmintic molecule. Hence there is an increasing demand towards natural anthelmintics. The helmentic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [4,5]

Uncaria gambir is known as safed kathha belongs to family Rubiaceae, a native Southeast Asian herbal plant, can mostly be found in countries such as Indonesia and Malaysia[6] which contains tannins namely, catechutannic acid, acacia catechin, catechu red, catechin, epicatechin & flavonoids - quercetin & quercitrin. [7] Gambirdine and isogambirdine also isolated from *Uncaria gambir*.[8] Many general traditional medicinal uses of *Uncaria gambir* include treatments for wounds and ulcers, fevers, headaches, gastrointestinal illnesses and bacterial/fungal infections.[9] It had been widely used as an astringent medicine for the treatment of spongy gums, tooth acne, diarrhoea and sore throat.[10] Besides being chewed, it is also imported in large quantities by the West for tanning, calico printing and dyeing purposes. [11] The earlier study have demonstrated potent anti-inflammatory activity, hypotensive effects[12] & antioxidant property.[13] It is evident that the plant has great potentials in treating various diseases. Thus, the present investigation was aimed to evaluate the anthelmintic activity of *Uncaria gambier* Roxb.

MATERIALS AND METHODS

Plant material

The dried leaves & shoots of *Uncaria gambier* Roxb. was procured form Yucca enterprises, Mumbai in the month of Jan. 2012 and it was authenticated by Prof. B. Amarendhar Reddy, Sai Gouthami College, Ibrahimpatnam, R.R.Dist, A.P, India.

Preparation of extracts

Dried leaves & shoots of *Uncaria gambier* Roxb. was coarsely powdered & extracted with absolute alcohol in soxhlet apparatus for 72 hrs. The liquid extract was filtered & then concentrated using rotary flash evaporator at a temperature less than 45^{o}C to get semisolid residue which was dried under vaccum. The dried extract was suspended in water and extracted with ethyl acetate which was again concentrated and dried. The alcoholic extract with its ethyl fractions were subjected for further studies.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out to confirm presence of tannins & flavonoids.[14]

Collection of worms

Indian adult earthworms *Pheretima posthuma* were collected from Sri Krishna Vermiculture Pvt. Ltd. Uppariguda, Ibrahimpatnam, Hyderabad. The earthworms were identified by Prof. J. Srikanth, Dept. of Zoology, Sri Chaitanya Junior College, Hyderabad. The average size of earthworms being 6-8 cm. Prior to experiment, they were washed with tap water for the removal of the adhering dirt.

Evaluation of anthelmintic activity

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.

Pheretima posthuma worms are easily available and used as a suitable model for screening of anthelmintic drug. The anthelmintic activity was carried as per method described by Panda *et al* with minor modifications. Both the test extracts & standard drug solution were freshly prepared before starting the experiment. The suspensions of test extracts were prepared in 2% gum acacia in distilled water to obtain dose of test drug at 25, 50, 75, 100 mg/ml. Albendazole suspension was also prepared in the same manner using 2% gum acacia in distilled water. The worms were divided into four groups each containing 6 worms. Grouping was done as follows:

Group I – Control (only the vehicle is used i.e. 2%w/v gum acacia)

Group II – Alcoholic extract treated

Group III – Ethyl acetate fraction treated.

Group IV- Standard drug (albendazole) treated.

Six worms were observed for their spontaneous motility and evoked responses. Time of paralysis is noted at different time intervals when no movement was observed except the worms were ascertaining that they neither moved even when shaken vigorously nor they revive even in normal saline. Time of death is noted when the worms showed zero response to the stimuli, even after performing the prick test and when dropped in warm water (50°C) followed with fading away of their body colour. All results were expressed as a mean ± SEM of six animals in each group. [15-17] The observation of test extracts & standard drug is shown in the table no. 1

RESULTS & DISCUSSIONS

From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed (Table no. 1). Although both the test extracts showed significant anthelmintic activity in a dose dependent manner but the ethyl acetate fraction appeared to be more effective. Evaluation of anthelmintic activity was compared with reference standard albendazole. The alcoholic extract showed time of paralysis & time of death as 6.01 & 10.20 min. whereas for ethyl acetate fraction it was 3.3 & 6.16 min. The reference standard albendazole showed the time of paralysis & time of death as 1.2 & 1.33 min. respectively.

Preliminary phytochemical analysis of test extracts revealed the presence of tannins & flavonoids. Tannins have been reported to produce anthelmintic activities[18-19] as they can bind to free proteins in the gastrointestinal tract of host animal[20] or glycoprotein on the cuticle of the parasite and thereby cause deaths[21]. The potent wormicidal activity of ethyl acetate fraction against earthworms suggests that it is effective against parasitic infections of humans.

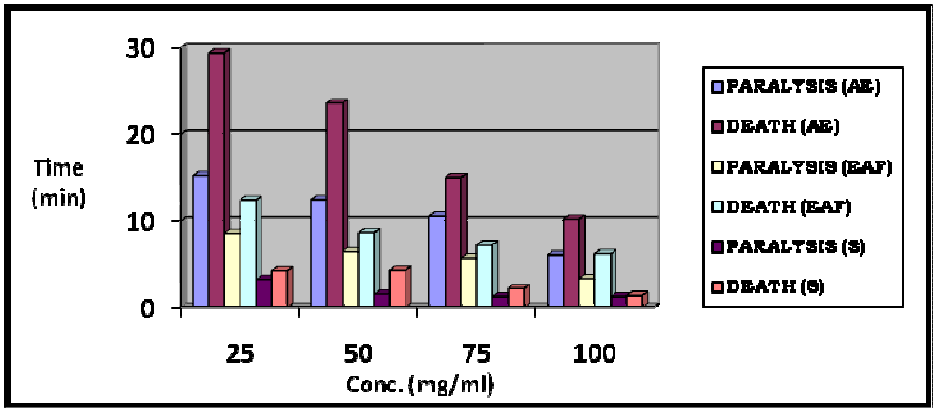
CONCLUSION

The ethyl acetate fraction of alcoholic extract of leaves & shoots of *Uncaria gambier* Roxb. exhibited significant anthelmintic activity against earthworms in dose dependent manner. The observed activity may be due to their phenolic content (flavonoids) which is worth for further investigations on isolation of the specific constituents.

Table No.1: Anthelmintic activity of test extracts of *Uncaria gambier* Roxb.

Test Drug	Conc. (mg/ml)	Paralysis Time (min.)	Death Time (min.)
Alcoholic Extract (AE)	25	15.22±0.16	29.4±0.07
	50	12.43±0.08	23.58±0.05
	75	10.54±0.13	15±0.11
	100	6.01±0.11	10.20±0.05
Ethyl Acetate Fraction (EAF)	25	8.5±0.08	12.4±0.07
	50	6.4±0.6	8.6±0.05
	75	5.67±0.06	7.2±0.11
	100	3.3±0.05	6.16±0.06
Albendazole (S)	25	3.2±0.01	4.17±0.01
	50	1.5± 0.01	3.23±0.10
	75	1.20±0.008	2.15± 0.01
	100	1.20±0.06	1.33± 0.06

Figure No.1: Histogram showing anthelmintic activity of test extracts of *Uncaria gambier* Roxb.



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