

Anthelmintic Evaluation of *Eichhornia crassipes* and *Tecoma stans* Flower ExtractsPrasanth M. Kumar^{1*}, Venkatesan Suba², Rami B. Reddy³, Srinivasa P. Babu¹¹Vignan Pharmacy College, Vadlamudi, Guntur, Andhra Pradesh, India.²Department of Pharmacology, National Institute of Siddha, Chennai, India.³Director-Formulations, NATCO Pharma, Hyderabad, Telangana, India.

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ABSTRACT

Eichhornia crassipes and *Tecoma stans* are used in folklore medicine for the treatment of various ailments including helminthiasis. The anthelmintic potential of different extracts prepared from *E. crassipes* and *T. stans* were tested on adult Indian earthworm (*Pheretima posthuma*). Two concentrations (100 mg/mL and 200 mg/mL) of each extract were used in the experiment, which involved the determination of the time of paralysis and time of death of the worms. Albendazole (10 mg/mL) and distilled water were included in the study as standard reference drug and control, respectively. Among all the extracts tested, the ethanol extract of both plant flower extracts under investigation showed better anthelmintic activity. The results of the present investigation support the traditional use of the two plants as anthelmintic agents.

Keywords: *Eichhornia crassipes*, *Tecoma stans*, *Pheretima posthuma*, anthelmintic activity.

Introduction

Helminth infections are one of the major health issues affecting billions of people worldwide especially in tropical and sub-tropical countries with low per capita income and poor hygiene conditions.^{1,2} These infections cause a huge economic loss in the form of impaired performance and reproduction, and sometimes significant weight loss and mortality.³ In most countries, synthetic anthelmintics are most widely used for the control of helminths. The synthetic anthelmintic drugs widely used today generally have side effects and teratogenic effects, for example, mebendazole has teratogenic effects in animals, hence it is contraindicated in pregnant women and not used in children below two years.⁴ Considering the adverse effects of synthetic drugs, researchers are now screening traditional medicinal plants for their anthelmintic activity. In tropical developing countries like India, traditional medicines are very popular and people have been consuming several plants or plant-based preparations for the treatment of helminthic infections.⁵

Eichhornia crassipes (Mart.) Solms, a native of South America, is one of the free floating macrophytes found in the aquatic environment such as ditches, ponds and lakes. It is universally called Water hyacinth. In folklore medicine, *E. crassipes* has been used to ease swelling, burning, haemorrhage, and goiters. In veterinary medicine, it has been used as a tonic for the skin of horses, for irritation and inflammation.⁶ The plant has been traditionally used in East Godavari district for the treatment of helminthic infections. The roots have been reported to have anthelmintic activity.⁷ However no literature is available on anthelmintic activity of *E. crassipes* flowers.

Tecoma stans (L.) from *Bignoniaceae* family is an ornamental plant found throughout India. The entire plant possesses medicinal value and is used for the treatment of various ailments. Flower infusion can be taken orally for diabetes and stomach pains.⁸ A strong flower and root decoction are taken orally as a diuretic, to treat syphilis or for intestinal worms. The plant is considered in the Satara District an effective remedy for snake and rats bites and for scorpion sting, as diuretic, vermifuge and tonic.⁹ Leaves of *T. stans* have been reported to have anthelmintic activity.¹⁰ However, no literature is available on the anthelmintic activity of *T. stans* flowers. The aim of the present study was to investigate the anthelmintic activity of different extracts prepared from *E. crassipes* and *T. stans* flowers.

Materials and Methods

Drugs and chemicals

All organic solvents and chemicals were of analytical grade. Albendazole was purchased from Bandy Mankind Pharma Ltd., New Delhi.

Collection and extraction of the plant materials

Flowers of *E. crassipes* and *T. stans* were collected in the month of June 2016 from Tirupathi and authenticated by Dr. Madhava Chetty, S.V. University, Tirupathi. The voucher specimens were deposited at the institution herbarium with voucher numbers 2140 and 1134 for *E. crassipes* and *T. stans*, respectively. The flowers were shade-dried and 100 grams of the dried powder was extracted in a Soxhlet extractor successively using 2 L of petroleum ether, ethyl acetate and ethanol. All the extracts were vacuum-dried to obtain petroleum ether extract, ethyl acetate extract and ethanol extract, respectively. The different extracts of *E. crassipes* were named as PEEC (petroleum ether extract of *E. crassipes*), EAEC (ethyl acetate extract of *E. crassipes*), and EEEC (ethanol extract of *E. crassipes*). The different extracts of *T. stans* were designated as PETS (petroleum ether extract of *T. stans*), EATS (ethyl acetate extract of *T. stans*), and EETS (ethanol extract of *T. stans*).

Phytochemical screening

Qualitative chemical tests to identify the phytochemicals were carried out on the various flower extracts using standard procedure.¹¹

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Anthelmintic activity

The anthelmintic activity was performed on adult Indian earthworms (*Pheretima posthuma*) due to its physiological and anatomical resemblance with the intestinal roundworm parasite, *Ascaris lumbricoides* of human beings.^{12,13} The earthworms (8-10 cm long) were collected from the moist soil as well as waterlogged area of Guntur district and authenticated by a zoologist. The different concentrations (100 and 200 mg/mL) of the extracts were prepared by triturating the sample in distilled water. Control was maintained using distilled water in one petri dish. All the test solutions and standard drug solutions were prepared freshly before starting the experiments. Formulation (50 mL) of different concentrations of the successive extracts were placed in petri dishes and earthworms were released into the respective petri dishes. All petri dishes were placed at room temperature. For each plant extract group, six earthworms were used and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. The death time of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. Death was concluded when the worms lost their motility followed with fading away of their body colors.¹⁴

Statistical analysis

The data are presented as the mean \pm standard error of mean (n = 6). Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software Version 20 (IBM). The significance of differences was defined at p < 0.05 and p < 0.01.

Results and Discussion

Due to the high cost of *in vivo* tests, *in vitro* tests have been used for initial screening of plant extracts for their anthelmintic activity.¹⁵ The anthelmintic activity was evaluated by using *Pheretima posthuma* as the experimental model. In the current study, the extracts of both plants evaluated have shown dose-dependent anthelmintic activity at different concentrations tested. The results of the anthelmintic activity of flower extracts of *E. crassipes* are shown in Table 1 and results for the flower extracts of *T. stans* are shown in Table 2. The ethanol flower extract of both plants has shown highly significant anthelmintic activity when compared to other extracts studied. In the untreated control group, no paralysis or death was noticed in earthworms even after a period of 24 h. The standard drug albendazole exhibited higher anthelmintic activity when compared to the plant extracts, paralysis time was found to be 2.70 \pm 0.18 and death time was found to be 4.31 \pm 0.23. At 100 mg/mL, with respect to PEEC extract, paralysis was observed at 26.20 \pm 0.67 min and death in 29.42 \pm 0.51 min, while EAEC showed paralysis and death in 14.26 \pm 0.61 min and 20.79 \pm 0.44 min, respectively. The EEEC showed paralysis and death in 8.47 \pm 0.42 min and 10.49 \pm 0.23 min, respectively. At 200 mg/mL concentration, the time required for paralysis with PEEC was 22.28 \pm 0.88 min and death in 24.58 \pm 0.35 min, while EAEC caused paralysis and death at 11.36 \pm 0.48 min and 16.07 \pm 0.42 min, respectively. Finally, EEEC caused paralysis and death at 7.71 \pm 0.30 min and 8.54 \pm 0.27 min.

As shown in Table 2, the extracts prepared from *T. stans* showed anthelmintic activity in a dose-dependent fashion. At 100 mg/mL, the time required for causing paralysis in case of PETS is 13.32 \pm 0.97 min and death in 16.60 \pm 0.39 min, while EATS caused paralysis and death in 9.79 \pm 0.43 min and 13.73 \pm 0.65 min, respectively. EETS showed paralysis in 6.64 \pm 0.42 min and death in 8.73 \pm 0.29 min. At 200 mg/mL concentration, the time required for causing paralysis in case of PETS is 10.95 \pm 0.61 min and death in 14.79 \pm 0.73 min, while EATS showed paralysis and death in 8.16 \pm 0.34 min and 11.24 \pm 0.20 min, respectively. At 200 mg/mL, EETS gave shorter paralysis time (5.94 \pm 0.45) and death time (7.11 \pm 0.33) when compared to other extracts studied. Benzimidazoles such as albendazole and mebendazole are the most common anthelmintic drugs used in human medicine. These drugs bind to intracellular tubulin, preferentially affecting parasites, thus inhibiting the formation of microtubules. This subsequently leads to disruption of cell homeostasis due to the impaired transport of secretory granules and enzymes in the cytoplasm. The mechanism of action of albendazole is by blocking glucose uptake in larval and adult stages of susceptible parasites, and also depleting their glycogen reserves, thus decreasing ATP formation.¹⁶ With an increase in the concentration of the flower extracts

(100-200 mg/mL), the time for the onset of death and paralysis was shortened. The probable reason for the observed differences in anthelmintic activity between the extracts could be due to variation in solubility of the active constituents in the three solvent systems used.¹⁷ Preliminary phytochemical screening of the ethanol extract revealed the presence of flavonoids, tannins and saponins. Flavonoids, tannins and saponins were shown to possess anthelmintic activity. Tannins are found to bind to free proteins in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and cause death.¹⁸ Saponins potentially act as anthelmintic agents by inhibiting the enzyme acetylcholinesterase, leading to worm paralysis and death. They affect the permeability of the cell membrane of worms and cause vacuolization and disintegration of tegument. Moreover, saponin can irritate the gastrointestinal mucous membrane channel of worms that interfere with the absorption of food.^{19,20} Flavonoids can inhibit larval growth and inhibit the arachidonic acid metabolism which may lead to the degeneration of neurons in the worm's body and eventual death.^{21,22} The presence of these phytochemicals may be responsible for the observed anthelmintic activity of the plant extracts in the present study.

Conclusion

The present study has shown that both plants examined have a promising *in vitro* anthelmintic activity against *Pheretima posthuma*. In view of these findings, further *in vitro* and *in vivo* evaluation is suggested including toxicity studies in order to exploit these plants as possible anthelmintic agents.

Table 1: Anthelmintic activity of *E. crassipes* flowers

Treatment	Paralysis time (min)	Death time (min)
Control (distilled water)	-	-
PEEC-100 mg/mL	26.20 \pm 0.67	29.42 \pm 0.51
PEEC-200 mg/mL	22.28 \pm 0.88	24.58 \pm 0.35
EAEC-100 mg/mL	14.26 \pm 0.61	20.79 \pm 0.44
EAEC-200 mg/mL	11.36 \pm 0.48	16.07 \pm 0.42
EEEC-100 mg/mL	8.47 \pm 0.42	10.49 \pm 0.23
EEEC-200 mg/mL	7.71 \pm 0.30	8.54 \pm 0.27
Albendazole-10 mg/mL	2.70 \pm 0.18	4.31 \pm 0.23

PEEC: Petroleum ether extract of *E. crassipes*; EAEC: Ethyl acetate extract of *E. crassipes*; EEEC: Ethanol extract of *E. crassipes*. Values are expressed as mean \pm SEM, (n = 6).

Table 2: Anthelmintic activity of *T. stans* flowers

Treatment	Paralysis time (min)	Death time (min)
Control (distilled water)	-	-
PETS-100 mg/mL	13.32 \pm 0.97	16.60 \pm 0.39
PETS-200 mg/mL	10.95 \pm 0.61	14.79 \pm 0.73
EATS-100 mg/mL	9.79 \pm 0.43	13.73 \pm 0.65
EATS-200 mg/mL	8.16 \pm 0.34	11.24 \pm 0.20
EETS-100 mg/mL	6.64 \pm 0.42	8.73 \pm 0.29
EETS-200 mg/mL	5.94 \pm 0.45	7.11 \pm 0.33
Albendazole-10 mg/mL	2.70 \pm 0.18	4.31 \pm 0.23

PETS: Petroleum ether extract of *T. stans*; EATS: Ethyl acetate extract of *T. stans*; EETS: Ethanol extract of *T. stans*. Values are expressed as mean \pm SEM, (n = 6).

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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