Original Article

Absence of anthelmintic activity of hydroalcoholic leaf extracts of *Artabotrys hexapetalus* (*Linn.f*)

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Abstract

Objective: To study the anthelmintic activity of *Artabotrys hexapetalus (Linn.f)* leaves. **Materials and Methods:** The extraction was made using the hydroalcohol with soxhlet extraction system. The hydroalcoholic extract of varying concentration was used for conducting anthelmintic activity (12.5, 25, 50 and 100 mg/ml). *Eudrilus eugeniae* (African adult earthworm) was used as the test organism. **Result:** The time of paralysis and death of the earthworms, the positive control (albendazole) was more effective and no effect was shown by the hydroalcoholic extract of varying concentrations. **Conclusion:** Hydroalcoholic extract of *Artabotrys hexapetalus*, didn't show any anthelmintic activity at the concentration of 12.5, 25, 50 and 100 mg/ml.

Key words: Albendazole, *Eudrilus eugeniae*, hyroalcohol, soxhlet extraction

INTRODUCTION

Medicinal plants have served through ages, as a constant source of medicaments for the treatment of a variety of diseases. The history of herbal medicine is almost as old as human civilization.[1] The World Health Organization (WHO) estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in the treatment of helminthes in humans.[2] Anthelmintics are the drugs or agents that destroy or cause the expulsion of parasitic intestinal worms. Helminthiasis is a macro parasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. It can have immunomodulatory effects on the host, with implications for any co-infecting pathogens.[3] An integrated approach is required for the effective control of helmintics, which includes strategic and tactical use of antihelmintics and careful management of grazing lands, including control of stocking rates and appropriate rotation strategies.[4]

Artabotrys hexapetalus (Linn.f) belongs to the custard apple family Annonaceae, consisting of trees and shrubs with about 2300-2500 species and more than 130 genera. *A. hexapetalus (Linn.f)* commonly known as Manoranjini is a powerful climber. The old stems of great thickness are covered with rather smooth grey bark and furnished with thick woody pointed spreading spines 2-4 cm long. The leafy branches are slightly puberulous. The flowers are very fragrant with yellow petals and its fruit is narrowly obovoid. The bark and roots are in general, used for dysenteries and as vermifuges, and leaves for dysenteries and fevers. Traditionally, decoctions of the leaves are used as a remedy for cholera and have been found to exhibit antifertility effects in rats.

Despite the arrays of documented reports of *A. hexapetalus (Linn.f)*, currently available literature revealed that there is a paucity of information on the potentials of this plant as a traditional remedy for intestinal helminthes.[5] As a part of research work efforts were made to investigate the *in vitro* anthelmintic activities of the hydroalcoholic extract of *A. hexapetalus (Linn.f)* leaves

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using adult African earthworm (*Eudrilus eugeniae*), which has similar anatomy and physiology to human intestinal helminthes.

MATERIALS AND METHODS

Collection and authentication of plant

The leaves of *A. hexapetalus (Linn.f)* maintained at Sugandhavana were collected from UAS (B), GKVK, Bangalore, Karnataka, India. The plant was identified and authenticated by Dr. M. Vasundhara, Professor, Division of Horticulutre, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka (No. 13/Hort/MADP [Authentication No. 1]).

Collection of worms

Worms required for evaluation of the anthelmentic activity of leaves of *A. hexapetalus (Linn.f)* were collected and authenticated by Prof. Govindraj, Department of Entomology, UAS (B), GKVK, Karnataka, India. Worms collected belonged to the genus *E. eugeniae* (African adult earthworm). The worms were placed in a ventilated bag with sufficient nutrients until the study was conducted.

Drugs and chemicals

In this study, albendazole was used as the Standard Drug (GlaxoSmithKline).[1] The concentration of standard drug was prepared in normal saline to give 15 mg/ml concentration. Normal saline was used during the experimental protocol.

Extraction process

Extraction is a process where the desired constituents of the plant are extracted using a solvent. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and the type of substance that is being isolated. Alcohol, in any case is a good all-purpose solvent for preliminary extraction. The classical chemical procedure for obtaining organic constituents from dry plant tissue (dried seeds, roots, and leaves) is through soxhlet apparatus using a wide range of solvents. The leaves were shade dried and made into coarse powder by using a mechanical grinder. The powdered material was packed in soxhlet apparatus and extracted with 80% (v/v) ethanol. The extract was concentrated and dried. The dried hydroalcoholic extract of *Artabotrys hexapetalus* (HAAH) was stored in an air tight container in the refrigerator at <10°C.

Experimental model

Adult African earthworms (*E. eugeniae*) of 5-8 cm in length, 0.1-0.3 cm in width and weighing 0.8-4.0 g were used for all experimental protocol due to their general anatomical and physiological resemblance with the intestinal roundworms parasites of human beings.[6] All the earthworms were sourced from moist soil within GKVK University campus and washed with normal saline to remove all fecal and waste matters. They were authenticated at the Department of Biological Sciences, UAS, GKVK, Bangalore, Karnataka, India.

Anthelmentic activity

A. hexapetalus (Linn.f) leaf extract were prepared at varying concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml. A volume of 10 ml of each concentration of hydroalcoholic extract was delivered into a petridish. Then six worms (same type) were placed in it. Similarly, for each concentration of hydro alcoholic extract, six worms were used. Time for paralysis was noted when the worm did not revive even in normal saline. Time for death of worms were also recorded when the worms lost their motility followed by fading away of their body color (when dipped in warm water of 50°C). Albendazole (15 mg/ml in distilled water) was used as a positive control.[5]

RESULTS

Data given in Table 1 revealed that the hydroalcoholic leaf extract of *A. hexapetalus (Linn.f)* (HAAH) did not show anthelmintic activity at any of the tested concentrations. Considering the time of paralysis and death of earthworms, the positive control (albendazole 15 mg/ml) was more potent than HAAH. The control (distilled water with tween 80) did not show any activity against earthworms.

DISCUSSION

The hydroalcholic leaf extract of *A. hexapetalus (Linn.f)* does not possess vermicidal property, which has been confirmed by the treatment of various concentration of the extract. Since the season of harvest might also have influenced leaves, which were harvested during the flowering season (February-April) there could be a possibility of translocation of metabolites from the source to the sink

Table 1: Anthelmentic activity of *Artabotrys hexapetalus (Linn.f)* leaf extract

Test group	Concentration mg/ml	Paralysis onset time (min)	Death time (min)
Control	-	-	-
Hydroalcoholic leaf extract of *Artabotrys hexapetalus (Linn.f)*	12.5	0	0
	25	0	0
	50	0	0
	100	0	0
Albendazole	15	6.15±0.02	53±0.08

that may be resulting in less concentration of phytochemical in the leaves.[7] Thus, it failed to display activity against the worms used in the study. Further research studies should be carried out using various species of organisms.

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