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Research Article

COMPARATIVE STUDIES ON IN VITRO ANTHELMINTIC ACTIVITY OF *GYMNEMA SYLVESTRE* AND *ACALYPHA FRUTICOSA FORSSK*

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ABSTRACT

The development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintics. Traditional medicines act as a source of easily available effective anthelmintic agent. In the current study, In vitro experiments were conducted on Indian adult earthworms (*Pheretima posthuma)* to determine the possible anthelmintic activity of crude methanol extracts of Gymnema sylvestre and Acalypha fruticosa forssk. Methanol extract of the two plants at different concentration (25, 50, 100, 200mg/ml) showed dose-dependent vermicidal activities and results were expressed in terms of time for death and time for paralysis of worms. Piperazine citrate was used as a reference standard at a concentration of 10 mg/ml. The present study revealed that the two plants possess potent anthelmintic activity when compared to Piperazine.

Keywords: Anthelmintic activity; Pheretima posthuma; Gymnema sylvestre; Acalypha fruticosa forssk.

INTRODUCTION

Helminthic infections are the most plebeian infections in human beings, recognized as much cause of chronic ill health and sluggishness affecting a large ratio of the world's population[1]. The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a consequent economic impact[2]. Due to limited availability and affordability of modern medicines most of the world's population depends on traditional medicinal plants to a greater extent. Traditional medicines act as a source of easily available effective anthelmintic agent to people with a broad spectrum of action like high percentage of cure with single therapeutic dose, cost effective and free from toxicity[3,4].

Gymnema sylvestre is an herb native to the tropical forests of southern and central India where it has been used as a natural medicine for treatment of diabetes for nearly two millennia[5]. Leaves of *G. sylvestre* are active against glycosuria and many other urinary disorders. The saponin gymnemic acid, constituent of the leaves, was shown to suppress sweet taste sensation and to inhibit glucose absorption in the small intestine[6]. Extracts of Gymnema is not only claimed to curb sweet tooth's but also for treatment of various problems as hyperglycemia, obesity, high cholesterol levels, anemia, digestion and anti microbial activity[7].

Acalypha fruticosa Forssk. [Euphorbiaceae] commonly known as 'Birch-leaved Acalypha' and 'Chinnichedi' is a strong smelling bushy shrub traditionally used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites[8]. Several pharmacological studies have revealed its antidiarrhoeal, antioxidant, anti-inflammatory, anticancer, antiplasmodial, wound healing and cytotoxic properties[9]. Quantitative estimation of phyto constituents present in the powdered samples of Acalypha fruticosa showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids[10].

MATERIALS AND METHODS

A. Fruticosa (whole plant) was collected from hill region of Marudhamalai near Coimbatore, Tamil Nadu, India, and authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India.

Gymnema sylvestre leaves were collected from palar river region of Vellore District, Tamil Nadu, and authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, and India.

Plant Extraction and processing

A. Fruticosa collected specimen was shade dried, powdered, sieved through 100 mesh range and extracted with methanol using a Soxhlet apparatus. The methanolic extract thus obtained was evaporated to dryness at 40º C under reduced pressure in a Rota vapor.

The shade-dried leaves of G. Sylvestre were powdered, sieved through 100 mesh range and extracted with Soxhlet apparatus using methanol. The extract thus obtained was evaporated to dryness at 40º C under reduced pressure in a Rota vapor.

Animals

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity of the plant extracts. The adult earthworms were collected from near by moist soil area and washed thrice with normal saline solution to remove all the fecal matter.

Table 1: Anthelmintic Activity of A.fruticosa and G.sylvestre

Plant extract	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
A.fruticosa	25	45.43±0.08	121.53±0.05
	50	32.60±0.07	92.43±0.04
	100	26.65±0.08	65.51±0.10
	200	20.40±0.06	57.68±0.08
G.sylvestre	25	40.48±0.08	127.51±0.10
	50	33.53±0.08	99.60±0.05
	100	27.36±0.05	82.01±0.001
	200	21.68±0.04	60.40±0.09

Peperazine citrate	10	23.23±0.06	63.31±0.06

Each value represents mean ± SEM (N=6). P<0.001significantly different compared with reference compound, Piperazine citrate, student's *t*-test.

Anthelmintic Activity

The anthelmintic assay was carried out as per the method explained by Ajaiyeoba et. Al[11,12]. The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity. Pheretima posthuma was placed in Petri dish containing 10ml of A. fruticosa and G. sylvestre methanolic extracts at four different concentrations (25, 50, 100, 200mg) each. Distilled water was used as control and piperazine citrate (10mg/ml) is used as reference standard drug. Each Petri dish was placed with 6 worms and observed for paralysis and 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 time death of worm (min) was recorded after ascertaining that worms neither moved when given external stimuli or when the worms lose their motility followed with fading away of their body color and the results were expressed in comparison to the standard drug Peperazine citrate at a concentration of 10mg/ml in Table 1.

Statistical Analysis

The results were analyzed for statistical significance using one way ANNOVA followed by student t-test. The P<0.001 was considered significant.

RESULTS AND DISCUSSION

By preliminary phytochemical screening it was found that A. Fruticosa contains alkaloids, tannins, phenols, steroids and flavonoids in high amount and G. Sylvestre contains oleanane type triterpenoid saponins, flavones, alkaloids and several acylated derivatives of deacylgymnemic acid.

The methanol extracts of G. Sylvestre and A. Fruticosa exhibited dose-dependent anthelmintic activities that caused paralysis at 45.2, 40.1 min (at 25mg/ml); 32.5, 33.3 min (at 50mg/ml); 26.7, 27.4 min (at 100mg/ml); 20.2, 21.6 min (at 200mg/ml). The earthworms were more sensitive towards G. Sylvestre and A. Fruticosa at 100 and 200 mg/ml concentrations as compared to the reference drug Piperazine citrate (10 mg/ml). P<0.001significantly different compared with reference compound, Piperazine citrate. Both the plants methanol extracts were more effective in causing the death of the worms as well as promoting paralysis and also found that the two plants possess potent anthelmintic activity when compared to Piperazine.

The reference standard Piperazine citrate causes paralysis of the worms so that they are expelled in the feces but the methanol extracts of the two plants not only demonstrated paralysis but also killed the worms. The anthelmintic activity of the methanol extracts of both the plants may be due to the presence of alkaloid compounds.

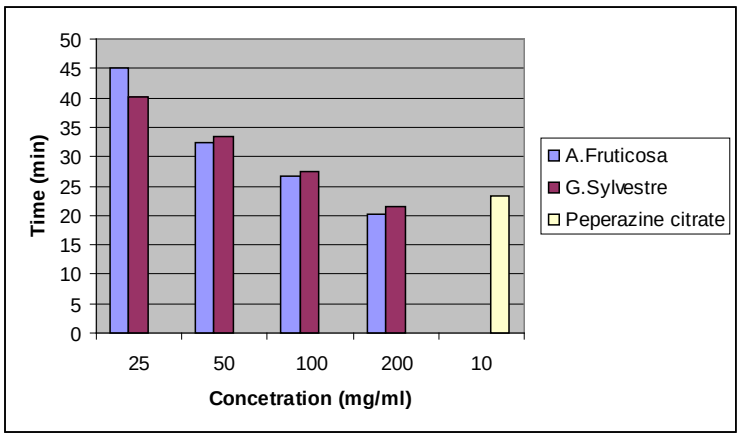

Fig. 1: Anthelmintic Activity of G. Sylvestre and A. Fruticosa Paralysis Time

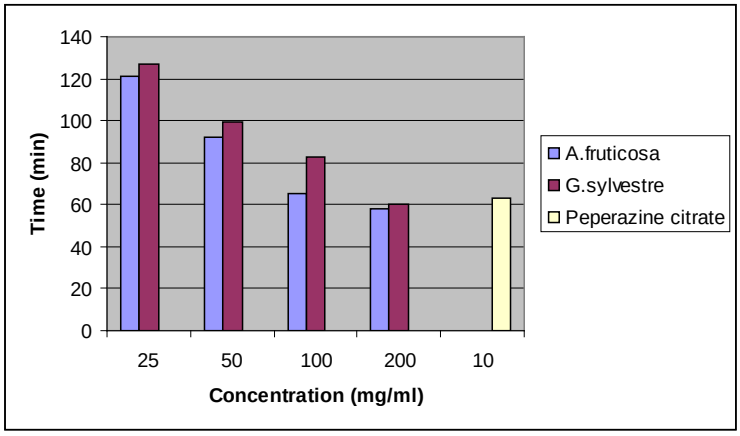

Fig. 2: Anthelmintic Activity of G. Sylvestre and A. Fruticosa Death Time

CONCLUSION

The anthelmintic activities of G. Sylvestre and A. Fruticosa have been tested against the Indian earth worm pheritima posthuma. It is concluded based on the findings of the present study that both the

plants possess varying degree of anthelmintic activity. Future scope involves isolation of phytoconstituents responsible for anthelmintic activity and study of its pharmacological actions.

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