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In vitro anthelmintic activity of *Gossypium herbaceum*

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Abstract

The present study was aimed at the evaluation of *in vitro* anthelmintic activity of ethanolic leaf extract of *Gossypium herbaceum* against Indian earthworm *Pheretima posthuma*. Three concentrations (25 mg/ml, 50 mg/ml, 75mg/ml) were tested and results were expressed in terms of time for paralysis and time of death of worms. In this study Albendazole was used as a standard drug. Ethanolic leaf extract of *Gossypium herbaceum* showed more significant activity at higher concentrations when compared to standard group (Albendazole).

Keywords: Anthelmintic activity, *Gossypium herbaceum*, Albendazole, Ethanolic extract, *Pheretima posthuma*

Introduction

Helminthes infections are among the most common infections in men, affecting a large proportion of the world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to those who visited these areas and some of them can develop in temperate climates [1]. Hence, the increasing prevalence of helminth parasites those are resistant to conventional anthelmintics has been the spur for different research programs exploiting alternative approaches to parasite control [2]. For much of our past history for ages, plant parts or entire plant extracts have been used to combat parasitism and in many parts of the world such natural products are still in use for this purpose.

From the ancient times, indigenous drugs have been used in the Indian medicinal system to treat different ailments and to provide therapeutic benefits. Our traditional system of medicine has made use of the different parts of plants in different types of diseases, including anthelmintic, anti-inflammatory and antimicrobial activities. Kavirajes and Hakims are still using several medicinal plants to treat helminthiasis. During the recent years, medicinal chemistry has made great strides, especially in synthetic chemistry but, for the sake of therapeutic effect up to the level and nontoxic treatment for helminthiasis, the research of plant-derived drug therapy is mostly needed [3].

In the present perspective of the developing countries, synthetic drugs are not only expensive and incapable for curing diseases but also have fatal adverse effects [4]. Thus, there is an urgent need to explore new antibacterial components with diverse chemical structures and novel mode of actions because of the increase in the incidence of new and re-emerging pathogenic diseases [5] to replace those which have lost their effectiveness.

Furthermore, we know our traditional and folkloric medicine has been using higher and aromatic plants for the purpose of extending the shelf life of foods showing inhibition against bacteria and yeasts [6]. Several studies have reported that many herbs possess varying degree of antimicrobial activities [7, 8]. Therefore, the natural medicinal plants may be a potent source of new antibacterial agents. *Gossypium herbaceum* L. belongs to Malveceae and commonly called as cotton plant [9, 10]. It is oldest Indian herbal drug, which is included in our present study is widely used by tribal people. Ayurvedic system has already noticed the importance of this plant. It has several experimentally proven pharmacological activities, which includes Antitumor [11], Antimutagenic [12], Anticonvulsant [13] antibacterial, anthelmintic [14] and antifungal activities [15]. Based on the literature review the present study was carried out anthelmintic activity of leaves of *Gossypium herbaceum*.

Materials & Methods

➤ Plant Collection

Gossypium herbaceum was collected in the month of November 2017 from Kagazimaddur village, narsapur, medakdist of Telangana, India.

➤ Preparation of plant extracts

The leaves of plant were dried under shade and crushed in pulverizer and powdered. The crude leaves extract was prepared by Soxhlet extraction method. 50g of powdered plant material was extracted with 500ml of solvent using ethanol and water individually. The process of extraction was carried out till the solvent in siphon tube of an extractor became colourless. The two extracts were filtered separately, the filtrates were placed in a beaker for evaporations. Further the dried extracts were maintained in a refrigerator at 4°C for further antiurolithiatic activity.

➤ Collection of Worms

Pheretima posthuma (earthworms) were collected from the manure and identified and washed with water to remove all kinds of dirty water from them.

➤ Chemicals and Drugs Used

Ethanol, Water, Normal saline, Albendazole

➤ Preparation of Concentrations

The ethanolic and Aqueous extracts of *Gossypium herbaceum* was made into four different concentrations such as 25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml by dissolving in normal saline. The standard control group Albendazole was prepared by using 0.5% w/v Carboxy Methyl Cellulose (CMC) as a suspending agent.

➤ Evaluation of Anthelmintic Activity

The anthelmintic activity was carried out according to the method [16]. The Indian earthworm (*Pheretima posthuma*) was placed in petri dish containing four different concentrations (25, 50, 75, 100mg/ml) of aqueous and ethanolic extracts of *Gossypium herbaceum*. Albendazole was used as a standard drug and observed for paralysis and death of worms. The lethal effect of Albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake [17]. Time for paralysis was noted when no movement of any sort could be observed except when worms were shaken vigorously. Death was concluded when the worms lost their motility [18, 19]. The results were compared with standard reference drug Albendazole treated samples.

Results and Discussion

Results

Table 1: Anthelmintic activity of Ethanol extract of *Gossypium herbaceum* and standard Albendazole.

Extract	Concentrations (mg/ml)	<i>Pheretima posthuma</i>	
		Paralysis (min)	Death (min)
Ethanol extract	25mg/ml	60±1.34	70±0.18
	50mg/ml	9±0.94	10±0.62
	75mg/ml	5±.09	6±0.57
Albendazole	25mg/ml	40±0.43	43±1.38
	50mg/ml	36±0.60	38±0.59
	75mg/ml	30±0.81	29±1.38
	100mg/ml	22±1.4	23±0.92



Fig 1: *In vitro* experimental model setup to evaluate antihelmintic activity



Fig 2: Antihelmintic activity of Ethanol extract of *Gossypium herbaceum*

Discussion

The earth worm *Pheretima posthuma* is one of the most important soil invertebrates in promoting soil fertility. These earthworms are important components of the diets of many higher animals. Helmenthic infections of the gastrointestinal tract of human beings and animals have been recognised too have adverse effect on health standards with a consequents lowering of resistance to other diseases. In search of components with anti helmenthic activity, a number of substances were screened using different species of worms, for example earth worm, ascaris, nippostrongylus and heterakia. Of all the species, earth worms have been used widely for the initial evaluation of antihelmenthic compounds *in vitro* because the resemble intestinal “worms” in their reaction to anti helmenthic and are easily available. It has been demonstrated that all antihelmenthics are toxic to earth worms and substance toxic to earth worms is worthy for investigation has an antihelmenthic. Ethanolic leaf extract of *Gossypium herbaceum* shows significant effect on *Pheretima posthuma*. Higher concentrations of ethanolic extract of *Gossypium herbaceum* extract produce paralytic effect much earlier and time taken for death was shorter. It shows maximum efficacy at 75mg/ml concentration than the standard drug (Albendazole) (Table 1).

Conclusion

In vitro antihelmintic activity has been performed on the selected plant *Gossypium herbaceum* by using the standard drug Albendazole. It can be concluded that the ethanolic leaf extract of *Gossypium herbaceum* has shown more significant anthelmintic activity when compared to Albendazole against Indian earthworm *Pheretima posthuma*. This study has given primary evidence for *Gossypium herbaceum* as the plant which possess anthelmintic property.

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