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In Vitro Anthelmintic Activity of *Chloris barbata*

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ABSTRACT

The present study was aimed at the evaluation of *in vitro* anthelmintic activity of ethanolic leaf extract of *Chloris barbata* against Indian earthworm *Pheretima posthuma*. Three concentrations (25 mg/ml, 50 mg/ml, 75 mg/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 *C. barbata* showed more significant activity at higher concentrations when compared to standard group (Albendazole).

KEYWORDS: Anthelmintic activity, *Chloris barbata*, Albendazole, Ethanolic extract, *Pheretima posthuma*

INTRODUCTION

The World Health Organization (WHO) reveals that over two billion people are suffering from parasitic worm infections [1]. It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced [2].

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 [3]. 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 [4].

Helminthiasis is a disease in which a part of the body is infested with parasitic worms like Roundworms (Nematodes), Tapeworms (Cestodes) or Flukes (Trematodes) (Rafi et al., 2011). Although the worms reside in the gastrointestinal tract, sometimes may burrow into the liver and other organs [5]. Drugs that either kill or expel infesting helminthes (Worms) are known as anthelmintic. Since ancient times the medicinal properties of plants have been investigated for scientific advancement throughout the world due to their potent anthelmintic activities [6,7]. Some broad spectrum anthelmintics (e.g. Piperazine citrate, Albendazole) are effective against parasitic flat worms as well as nematodes. However, majority of drugs are limited in their action (e.g. Praziquantel) as resistance may be developed very quickly [8,9] and also the toxicity problems may be occurred (Akhtar, et al., 2000). Therefore, it is necessary to find out new medicinal plants having broad spectrum anthelmintic activity with less toxicity [10,11].

Plants have provided man with all his needs in terms of shelter, clothing, food, flavours and fragrances. Plants have formed the basis of system among traditional medicine which has given rise to some important drugs still in use today. Many ancient nations have awakened to the importance of herbal medicine which brings more cures [12].

The exploration of new medicinal properties of various plant species has induced the attention of the scientists towards the biologically active compounds since the last couple of decades. The reason behind this is that the bioactive compounds possess potent pharmacological activities and have low or no toxicity [13]. This emerged interest to plant-derived medicines is mainly due to the resistance caused by indiscriminate use of synthetic medicines as well as the on-going perception that green medicines are safer than the synthetic drugs having severe adverse effects [14].

The selected plant *C. barbata* have occupied an important place in Indian culture and folk medicines. This plant has been extensively in Ayurvedic system of medicine and is used throughout India. The plant shows various pharmacological activities like Anti-diabetic [15], Anti-helminthic, Anti-pyretic, Anti-inflammatory, Analgesic, Antibacterial [16], etc.

MATERIALS AND METHODS

Plant Collection

Chloris barbata was collected in the month of November 2017 from Kagazimaddur village, Narsapur, Medak dist. 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. 50 gm of powdered plant material was extracted with 500 ml of solvent using ethanol. 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 extracts of *C. barbata* was made into three different concentrations such as 25 mg/ml, 50 mg/ml, 75 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 [17]. The Indian earthworm (*P. posthuma*) was placed in petri dish containing three different concentrations (25, 50 and 75 mg/ml) of ethanolic extracts of *C. barbata*. 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 [18]. 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 [19,20]. The results were compared with standard reference drug Albendazole treated samples.

RESULTS AND DISCUSSION

RESULTS

In my clinical practice, following up of 50 patients within one year, combination therapy of Vitamin E, Metformin and Milk Thistle found to be effective in decreasing liver steatosis.

Table 1: Anthelmintic activity of ethanolic extracts of *Chloris barbata* and standard Albendazole

Extract	Concentrations (mg/ml)	<i>Pheretima posthuma</i>	
		Paralysis (min)	Death (min)
Ethanolic extract	25 mg/ml	75 ± 1.34	85 ± 0.18
	50 mg/ml	17 ± 0.94	19 ± 0.62
	75 mg/ml	03 ± 0.09	04 ± 0.57
Albendazole	25 mg/ml	40 ± 0.43	43 ± 1.38
	50 mg/ml	36 ± 0.60	38 ± 0.59
	75 mg/ml	30 ± 0.81	29 ± 1.38



Figure 1: *In vitro* experimental model setup to evaluate anthelmintic activity



Figure 2: Anthelmintic activity of ethanol extract of *Chloris barbata*

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. Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized too have adverse effect on health standards with a consequent lowering of resistance to other diseases. In search of components with anthelmintic 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 anthelmintic compounds *in vitro* because they resemble intestinal "worms" in their reaction to anthelmintic and are easily available. It has been demonstrated that all anthelmintics are toxic to earth worms and substance toxic to earth worms is worthily for investigation has an anthelmintic. Ethanolic leaf extract of *C. barbata* shows significant effect on *P. posthuma*. Higher concentrations of ethanolic extract of *C. barbata* produce paralytic effect much earlier and time taken for death was shorter. It shows maximum efficacy at 75 mg/ml concentration than the standard drug (Albendazole) (Table 1).

CONCLUSION

In vitro anthelmintic activity has been performed on the selected plant *C. barbata* by using the standard drug Albendazole. It can be concluded that the ethanolic leaf extract of *C. barbata* has

shown more significant anthelmintic activity when compared to Albendazole against Indian earthworm *P. posthuma*. This study has given primary evidence for *C. barbata* as the plant which possess anthelmintic property.

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