In Vitro Anthelmintic Activity of Leaf Ethanolic Extract of Cassia Alata and Typha Angustifolia

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Abstract

Alcohol extract from the leaves of Cassia alata and Typha angustifolia were investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli. Various concentrations (10-100 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control.

Key Words: Anthelmintic, Cassia Alata, Typha Angustifolia, Pheretima Posthuma, Ascardia Galli

1. INTRODUCTION

Parasitoses have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance [1]. Gastrointestinal nematodes have been found to be of great economic importance in domesticated livestock throughout the world because of their adverse effects on productivity. Livestock producers have generally derived substantial benefits from the use of anthelmintics in controlling livestock parasitoses. In developing countries, small-scale farmers have a limited access to the commercially available anthelmintics and veterinary services either due to their non-availability or high costs. Most of the farmers, therefore, have to rely on the ethnoveterinary medicine as in some other parts of the world.

Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health [2]. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies on traditionally used anthelmintic drugs, which are generally considered to be very important sources of bioactive substances [3]. In the present study, anthelmintic potential of alcoholic extracts of leaves of Cassia alata, and Typha angustifolia have been evaluated.

1.1 Plant Collection and Authentication

Typha angustifolia (Family: Typhaceae is commonly known as Elephant grass or cattail. This plant is characterized by its fast growth and high biomass [4]. Several parts of the plant are edible, including dormant sprouts on the roots and bases of the leaves, ripe pollen, the stem and the starchy roots [5]. The traditional uses of pollen grains of T. angustifolia for treatment of kidney stones, abnormal uterine bleeding, abscesses, tapeworm infection diarrhea and dysentery is well known [6]. Modern research on pollen grains of T. angustifolia mainly reveals that it contain sterols, terpinoids, flavonoid glycosides [7], cerebrosides and long chain hydrocarbons that pocess various pharmacological activities like immunosuppression [8], antiplatelet aggregation [9], antimicrobial [10], cholesterol lowering activity and antiatherogenic effect [11]. The rhizome flour of T. angustifolia used in the treatment of human IBD is also studied [12]. All parts of the plant like leaves, seeds and roots have been traditionally used to relieve variety of ailments.

Cassia alata Linn. (Fam: Caesalpiniaceae) is a large handsome shrub with thick downy branches, found wild almost throughout India. Leaflets are 8-12 pairs, lower leaflet oblong-elliptic; upper ones broadly obovate. It is known as ringworm shrub and winged senna in English; Dadrughna and Dvipagsti in Sanskrit; semaiagathi and Vandugolli in Tamil [13]. In India systems if neducube, the leaves of the plant are used as purgative, expectorant astringent, vermicide and to treat all skin diseases. Extracts of C. alata leaves have been reported to possess analgesic, antibacterial, antiinflammatory, fungicidal, hypoglycemic, laxative, and oxytocic and wound healing activity etc. [14].

In Ayurveda, these plants are reported to be useful in jaundice, and in other traditional system of medicine are highly valued for treatment of various ailments [15]. C. alata and T. angustifolia leaves were collected from Yellagiri hills, Tamil nadu during October/November 2014. It was identified and authenticated by Dr. P. Jayaraman, Director Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen No. PARC/2013/2121.

1.2 Extract Preparation

The leaves were cleaned, shade dried and coarsely powdered. The coarse powder was then exhaustively extracted in a Soxhlet apparatus. 80% Ethyl alcohol was used as a solvent for alcoholic extract. The solvent was allowed to evaporate in a rotary vacuum evaporator. The dry extracts obtained were subjected to various chemical tests to detect the presence of different phytoconstituents [16]. Pheretima posthuma (Annelida), commonly known as earthworm were collected from the water logged areas and Ascardia galli (nematode)

worms were obtained from freshly slaughtered fowls (Gallus gallus). Both worm types were identified at the P. G. Department of Zoology, Presidency College, Chennai.

2. METHODOLOGY

The anthelmintic assay was performed according to the method of Ajaiyeoba et al., 2001 [17] with minor modifications. The assay was performed on adult Indian earthworm, P. posthuma due to its physiological resemblance with the intestinal roundworm parasite of human beings [18]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro [19]. Ascardia galli worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was advocated earlier [20].

Fifty millilitre of extract containing three different concentrations, each of crude alcoholic extract (10, 50 and 100 mg/ml in distilled water) were prepared and six worms were placed in it. This was done for both types of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Mortality time of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50° C). Piperazine citrate (10 mg/ml) was used as reference standard and distilled water as control [21].

3. RESULTS AND DISCUSSION

Preliminary phytochemical screening of alcoholic extract revealed the presence of carbohydrates, flavonoids, phenolic compounds, sterols and terpenoids in T. angustifolia and glycosides and flavonoids in C. alata. The alcoholic extracts of leaves of C. alata, T. angustifolia displayed significant anthelmintic properties at higher concentrations. The extract showed anthelmintic activities in a dose-dependant manner with 100mg/ml showing maximum effects against both the worms shortest time of paralysis and death with 100mg/ml concentration, for both type of worms. The alcoholic extract of C. alata, caused paralysis in 8 min and death in 28 min, while T. angustifolia extract showed paralysis and death in 10 and 30 min. against the P. posthuma. The reference drug piperazine citrate showed the same effects at 21 and 59 min respectively. Ascardia galli worms also showed sensitivity to the extracts of C. alata and T. angustifolia. The alcoholic C. alata extract caused paralysis in 5 min, death in 29 min and the T. angustifolia extract displayed paralysis and death in 6 and 27 min, respectively, at the concentration of 100 mg/ml. Piperazine citrate exhibited similar results the same at 12 and 41 min (Table 1).

The predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [22]. The leaf extract of T. angustifolia not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug piperazine citrate. Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituent.

Table 1. *In vitro* anthelmintic activity of leaf ethanolic extract of *Cassia alata* and *Typha angustifolia*

GROUPS	Conc (mg/ml)	Pheretima posthuma		Ascaridia galli	
		Paralysis (P) in min.	Death (D) in min.	Paralysis (P) in min.	Death (D) in min.
CONTROl	(0.5% CMC)	----	-----	-----	----
STD	(Piperazine citrate 10 mg/ml)	21.42 ± 2.35	59.10 ± 3.51	12.14 ± 0.62	41.88± 2.30
EECA	10 mg/ml	---	---	---	---
	50 mg/ml	42.07 ± 1.88	68 ± 2.00	20.00± 0.74	60.31± 2.86
	100 mg/ml	8.16 ± 0.85**	28.23 ±1.11	5.02± 1.04**	29.22± 0.52**
EETA	10 mg/ml	---	---	---	---
	50 mg/ml	48.52 ± 2.15	74.78 ±2.54	12.00± 0.18	57.26± 1.14
	100 mg/ml	10.12 ± 0.65**	30.09 ±0.98**	6.25 ± 0.45**	27.55± 1.23**

** Values represent Mean± SEM; n=5.
Comparisons made between standard Vs test groups,
P<0.05 was considered significant

Tannins were shown to produce anthelmintic activities [23]. Chemically tannins are polyphenolic compounds [24]. Some synthetic phenolic anthelmintic e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [25]. It is possible that tannins contained in the extracts of T. angustifolia and Cassia alata produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal [26] or glycoprotein on the cuticle of the parasite [27] and cause death.

4. CONCLUSION

In conclusion, the traditional claim of leaves of T. angustifolia and C. alata as anthelmintic have been confirmed as the leaf extracts displayed activity against the worms used in the study. Further studies to isolate and reveal the active compounds contained in the crude extracts of T. angustifolia and C. alata and to establish the mechanisms of action are required.

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