

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF *INDIGOFERA TINCTORIA* LINNGUNASEKARAN BALAMURUGAN*¹, SHINNARAJ SELVARAJAN²

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ABSTRACT

Extracts from the whole plant of *Indigofera tinctoria* Lin were investigated for their anthelmintic activity against *Pheretima posthuma*. Various concentrations (50 and 100 mg / ml) of each extract were tested in the assay, which involved the determination of paralysis time and death time of the organisms. The methanol extract exhibited a maximum anthelmintic activity comparable to standard drug Piperazine citrate (10 mg/ml). The petroleum ether and chloroform extracts exhibited a modest activity. The Preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts..

KEY WORDS: *Indigofera tinctoria*, *Pheretima posthuma*, Anthelmintic assay, Phytochemical screening, Tannin.

INTRODUCTION

Helminthes are recognized as a major problem to livestock production throughout tropics^[1]. Most diseases caused by helminthes are of a chronic and debilitating in nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity^[2]. Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites^[3]. Herbal

drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance^[4, 5]. As a step in this direction we focused our attention on search of herbal remedy and selected a medicinal; plant named *Indigofera tinctoria* Linn.

Indigofera tinctoria Linn [Family Papilionaceae] is a medicinal plant extensively used for its blue dye Indigo. It is a small erect shrub cultivated in all parts of India especially in Southern India TamilNadu^[6]. The plant is a purgative, antiseptic and astringent. The plant lessens inflammation, cures chronic bronchitis and asthma, cures piles, leucoderma, bites of insects and reptiles and enlargement of liver and spleen. In Ayurveda and Siddha systems, the entire plant is used for the treatment of

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helminthic infections ^[7-9]. So the present work was conceived by us to evaluate the anthelmintic activity of the entire plant of *Indigofera tinctoria* and substantiate the folklore claim.

MATERIALS AND METHODS

Plant Material: The whole plant of *Indigofera tinctoria* was collected in the month of October 2009 from Thirunelveli District of Tamil Nadu, India. The collected plant with complete herbarium was authenticated by Botanical Survey of India, Southern Circle, Tamil Nadu. [BSI]. A sample specimen was deposited in C. L. Baid Metha College of Pharmacy for future reference. The plant was dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction.

Preparation of Crude extract: Different solvents like petroleum ether, chloroform and methanol were chosen for successive solvent extraction based on polarity using soxhlet extraction apparatus and the extracts were concentrated under reduced pressure using rotary evaporator.

Preliminary phytochemical screening: Preliminary phytochemical screening was carried out by standard methods ^[10]. The screening covered mainly alkaloids, glycosides, sterols, terpenes, flavanoids, saponin, tannins, protein and reducing sugar. The presences of phytoconstituents are reported in Table 1.

Anthelmintic Assay: The anthelmintic assay was carried out as per standard method with minor modifications ^[11]. The assay was performed on adult Indian earth worm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings ^[12-15]. Because of easy availability, earthworms have been used widely for the evaluation of anthelmintic compounds in vitro ^[16-19]. All the extracts were dissolved in minimum amount of dimethyl sulphoxide and then volume was adjusted with saline water. 50 ml of formulation containing four different

concentrations of each of the extract (50 and 100 mg / ml in normal saline) were prepared and 10 worms (same type) were placed in it. The drug and extract solutions were prepared freshly before starting the experiment. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed by fading away of their body colours. Piperazine citrate (10 mg / ml) was used as reference standard. The results are reported in Table 2.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, alkaloids, tannins, flavanoids and steroids. As shown in the table 2, the methanolic extract of *Indigofera tinctoria* displayed a significant anthelmintic property in a dose dependent manner giving shortest time of paralysis and death with 100 mg/ml concentrations, comparable with the standard drug. The petroleum ether and chloroform extracts exhibited a modest activity (Fig 1). Phytochemical analysis of the crude extract revealed presence of tannins as one of the phytoconstituent. Tannins were shown to produce anthelmintic activities ^[20]. Chemically tannins are polyphenolic compounds ^[21]. Some synthetic phenolic anthelmintics e.g. niclosamide, oxiclosamide and bithinol are shown to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation ^[22]. It is [possible that tannins contained in the extracts produced similar results. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal ^[23] or glycoprotein on the cuticle of the parasite and cause death ^[24]. Results reported in the resent work constitute rational evidence and a scientific basis to justify and support the folklore claims of the potential anthelmintic activity of

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Indigofera tinctoria Linn. Further study is required to isolate the active constituents for the development of novel standardized anthelmintic herbal formulations.

Table 1: Qualitative analysis of various extracts of *Indigofera tinctoria*

Phytoconstituents	Petether	Chloroform	Methanol
Alkaloids	-	-	+
Carbohydrates	-	+	+
Flavanoid	-	-	+
Glycosides	-	+	+
Proteins	-	-	+
Saponin	-	-	+
Steroids	+	-	-
Tannin	-	-	+
Terpenoids	-	-	+

Table 2: Anthelmintic activity of *Indigofera tinctoria*

Treatment Group	Conc. (mg/ ml)	Paralysis time (min)	Death time (min)
Normal saline	-	-	-
Pet. Ether Extract	50	69.2±0.8	81.1±0.9
	100	52.1±0.9	67.9±1.3
Chloroform Extract	50	67.2±1.3	73.7±0.5
	100	53.7±0.9	61.1±0.5
Methanol Extract	50	32.6±1.2	40.6±0.9
	100	27.3±0.5	33.4±1.2
Piperazine citrate	10	25.6±0.32	28.6±0.8

Values are expressed as Mean ±SEM, n=6.

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Source of Support: NIL
Conflict of Interest: NONE