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In vitro anthelmintic activity of *Hugonia mystax* Leaves Linn in Indian Adult Earthworm

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Abstract
Background: To evaluate the *In-vitro* anthelmintic activity of ethanolic extract of *Hugonia mystax* leaves linn in Indian Adult earthworm.
Results: The phytochemical analysis of ethanolic extract of *Hugonia mystax* showed the presence of carbohydrates, flavonoids, steroids, saponins, terpenoids and absence of alkaloids, proteins and amino acids. All the investigational extract acquired the Anthelmintic activity at the minimal dose of 25 mg/ml its significant activity ($p<0.05$) at 25 mg/ml for time taken to paralysis and death when compared to the standard drug Alendazole 20 mg/ml respectively.
Conclusion: Based on the study, we conclude that ethanolic extract of *Hugonia mystax* leaves linn has the maximum Anthelmintic activity potential and can be employed in human helminth infections.

Keywords: Anthelmintic, earthworm, *Hugonia mystax*, paralysis, Alendazole.

1. Introduction
Helminth infections are among the most common infections in man, 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 travelers who visited those areas and some of them can develop in temperature climates [1]. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm, or tapeworm. Typically the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs; infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation [2].
The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India [3, 4]. This plant *Hugonia mystax* is locally known as Modirakanni. Ethnobotanically, the fruits are used by the tribals of Kalakad Mundanthurai for the treatment of Rheumatism [5]. Roots of *Hugonia mystax* were evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids.
Taking into consideration of medicinal value and utility, the present study was planned to explore the anthelmintic activity of ethanolic extract of the medicinal plant named *H. mystax*.

2. Materials and Methods
2.1 Collection, identification and preparation of plant materials
Fresh leaves of *H. mystax* were collected from velliangiri hills from Coimbatore DT. It was identified by a scientific officer, Dr. P. Samydurai Asst. Prof. Department of Botany Kongu Nadu arts and science college Coimbatore.

2.2 Preparation of Extracts
Leaves of *Hugonia mystax* (figure 1) were dried in the shade for two weeks. Dried leaves were coarsely powdered, sieved (#40) and stored in an airtight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and ethanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator.
The yields of various extracts were found to be 4.5% w/w (petroleum ether), 4.7% w/w (chloroform) and 10.5% w/w (ethanol). All the extracts were preserved in a refrigerator at 4 ℃. However, only ethanolic extract of the leaves was selected for further studies.

Fig 1: Leaves of *Hugonia mystax*

The linaceae is a relatively small plant family that consists of only three genera, among which is the genus *Hugonia* in which two species namely *Hugonia mystax* Linn and *Hugonia ferruginea* Wight & Arn are found in India. Leaves 3.8-6.3 by 2.5-3.8 cm, elliptic –obovate, obtuse or subacute, entire reticulately veined, the veins conspicuous on both surfaces, glabrous, base tapering, petioles 1.5 mm long hairy, stipules lanceolate-subulate.

2.3 Phytochemical Analysis

The phytochemical analysis of ethanolic extract of *Hugonia mystax* showed the presence of carbohydrates, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins and amino acids.

2.4 Experimental Model Indian Earthworm

Pheretima posthuma was used to study the Anthelmintic activity. Earthworms were obtained from moist soil near Erode DT. The worms are washed with normal saline to remove all the fecal matter and waste surrounding body.

The earthworms *Pheretima posthuma* 3-5 cm in length and 0.1-0.2 cm in width weighing 0.8-3.04 g were used for all experimental protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence where used to study the Anthelmintic activity [6-7].

2.5 Anthelmintic Investigation

Indian adult earth worm 4 -5 cm in length and 0.1 - 0.2 cm in width was used for the *in vitro* anthelmintic bio assay of ethanolic extracts. The worms were divided into the respective groups containing six-earth worms in each group. All the prototypes were dissolved in minimum quantity of 0.5% v/v CMC and the volume was adjusted to 10 ml with normal saline for making the concentration of 10, 20 and 50 mg/ml. All the prototypes and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed with normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (normal saline), and Albendazole (20 mg/ ml) and prototypes (25, 50 and 100 mg/ml) the anthelmintic activity was determined. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color. Six worms of about the same size per petridish were used. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms.

3. Results

Each crude extract containing 25, 50 and 100 mg/ml, produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. As evident from the available literature, Anthelmintic activities of all prototypes were tested in this bioassay at various concentrations of 25, 50 and 100 mg/ml (Table 1).

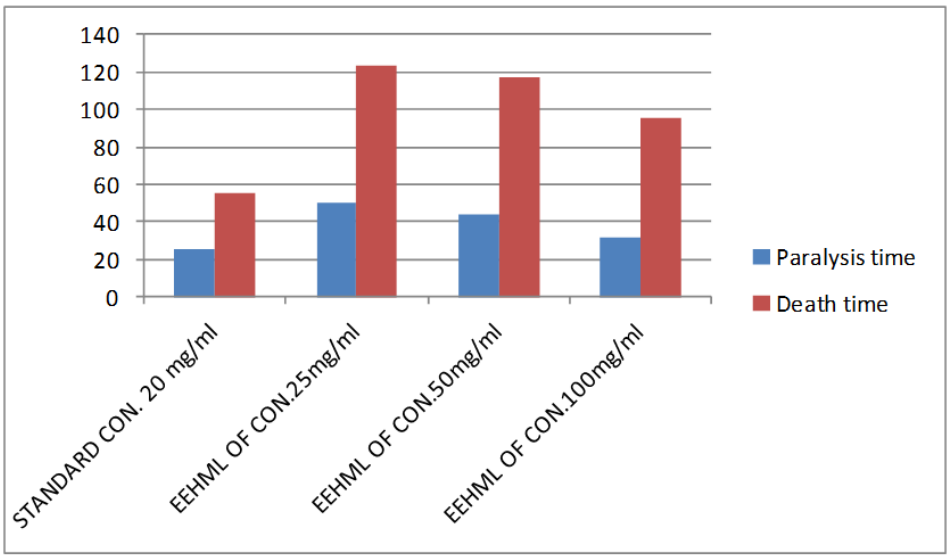

Fig 2: Activity of Ethanolic Extract of *Hugonia Mystax* Leaf

Table 1: Anthelmintic Activity of Ethanolic Extract of *Hugonia Mystax* Leaf

Extract	Concentration(mg/ml)	Pheretima posthuma	
		Paralysis time (minutes)	Death time (minutes)
Control (0.5%CMC)	-	-	-
Standard (Albendazole) 20 mg/ml	20 mg/ml	25.8±6.2	55.2±4.4
Ethanolic Extract of *Hugonia mystax* Leaves (EEHML)	25 mg/ml	50.4±8.4	123.7±14.2
	50 mg/ml	44.6±5.2	117.3±9.6
	100 mg/ml	32.5±2.8	63.9±8.6

Values are mean±SD, n=6

4. Discussion

Preliminary phytochemical studies on *Hugonia mystax* revealed the presence of flavanoid glycosides, steroids, carbohydrates, tannins, proteins and flavonoids. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity.

From the result ethanolic extract of *hugonia mystax show* an anthelmintic activity when compared to the standard drug. Each crude extract at the concentration of 25, 50 and 100 mg/ ml produced anthelmintic activity in a dose dependent manner, giving the shortest time of paralysis (P) and death (D) with 100 mg/ml concentration.

Phytochemical analysis of the crude extracts revealed the presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activities [8]. Chemically tannins are polyphenolic compounds [9]. Some synthetic phenolic anthelmintics (eg) niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [10]. It is possible that tannins contained in the extracts of *hugonia mystax* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal [11] or glycoprotein on the cuticle of the parasite [12] and cause death.

5. Conclusion

The traditional claim of aerial parts of *hugonia mystax* as an anthelmintic has been confirmed as the extracts shown activity against *Pheretima posthuma*. Further studies are necessary to isolate and reveal the active compound contained in the crude extracts of *hugonia mystax* responsible for activity and to establish the mechanism of action are required.

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