Anthelmintic activity of stem bark extract of *Hugonia mystax* Linn in *Pheretima posthuma* - An in vitro Study

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Abstract
The aim of the present study was to evaluate the anthelmintic activity of stem bark extract of *Hugonia mystax* Linn in *Pheretima posthuma*. The preliminary 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. The ethanolic 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 10 mg/ml respectively. Based on the study, that ethanolic extract of stem bark of *Hugonia mystax* Linn has the maximum Anthelmintic activity potential and can be employed in human helminthes infections.

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

INTRODUCTION
Helminth infection is one of the major public health problems, in more tropical countries. Helminthes infections are being recognized as a cause of acute as well as chronic illness among the various human beings as well as animals. Anthelmintic are drugs to that expel parasitic worms from the body, by either stunning or killing them. The most of drugs available for treat disease. Therefore, there is a scope for search of new drugs for the treatment of worm infection especially from the herbal origin, which is known to possess insignificant side effect and enhanced potency.

The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India [1-2]. Bruised roots are employed externally in reducing inflammatory tumours and as an antidote to snake bites [3-5]. Roots were used as anthelmintic, astringent and also used for dysentery, fever, inflammation, and rheumatism. Form of a powder it is administered internally as an anthelmintic and febrifuge. Bark of the root is employed as an antidote to poison. Used for skin diseases by the traditional healers of Tiruvannamalai hills, Tamilnadu [6-7]. Ethnobotanically, the leaves are used for anthelmintic and rheumatism. Ethnobotanically the bark is made in to a decoction with Curcuma aromatic and is given with honey for inflammations in stomach, vomiting, stomach pain, indigestion. The aerial parts used as herbal remedies for diabetes (10). Totally 62 chemical compounds were identified from stem of *Hugonia mystax*. Di-n-octyl phthalate (24.32%), 2-methyl-7-nonadecene (20.83%), α-D-Glucopyranoside, methyl (21.10%) were major constituent with the biological activities like antimicrobial, antifungal and antioxidant activity present in the stem extracts(10).Twenty compounds were identified from bark of *Hugonia mystax*. 2- Furan carboxaldehyde, 5– (hydroxyl methyl) – (27.64%), à-D-Glucopyranoside, methyl, (15.00%), n-Hexadecanoic acid (14.69%), 9,12-Octadecadienoic

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acid (Z,Z)- (7.24%), Oleic Acid (7.03%), Benzaldehyde, 2-hydroxy-6-methyl- [Synonyms: 2,6-Cresotaldehyde] (6.79%), Benzofuran, 2,3-dihydro-[Synonyms: Coumaran] (5.25%), Octadecanoic acid (2.24%), 1-Docosene (1.69%) and Stigmasteran-6,22-dien, 3,5-dihydro- (1.49) [8]. Pharmacological studies such as anti-inflammatory, antimicrobial, antioxidant, cancer prevention, Nematicide, Hypo-cholesterolemic, anti-tumour, Immuno-stimulant, Diuretic [3, 6-10] and Anti-diabetic [11-12] were reported.

The aim of present study to investigate the anthelmintic activity of ethanolic extract of stem bark of *Hugonia mystax* Linn against *Pheretima posthuma*.

**MATERIALS AND METHODS**

**Plant materials**

Fresh stem bark of *H. mystax* was collected and authenticated by the Botanist Dr. K.Madhavachetty, Department of Botany, Sri Venkateshwara University, Tirupathi.

**Preparation of Extracts**

The dried powdered plant material was extracted with ethanol using soxhlet apparatus. The extracts were concentrated to dryness using rotary flask evaporator. A greenish semi-sloid mass obtained and stored in a refrigerator at 4 °C for further use. The ethanolic extract of the stem bark was used for anthelmintic activity.

**Phytochemical Analysis**

The extracts of stem bark of *Hugonia mystax* were subjected to preliminary phytochemical analysis using standard protocol [13, 14].

**Anthelmintic activity**

The stem bark extract of *Hugonia mystax* were evaluated for anthelmintic activity in *Pheretima posthuma* (Indian adult earth worm) 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 earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence where used to study the Anthelmintic activity [15-16]. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into five groups of six earth worms in each and placed in eight Petri dishes containing the extract solutions or the reference drugs as mentioned below-

<table>
<thead>
<tr>
<th>Experimental Grouping of Earthworms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group -1: Received normal saline which served as control.</td>
</tr>
<tr>
<td>Group-2: Received Albendazole suspension at a dose of 10mg/ml which served as the standard.</td>
</tr>
<tr>
<td>Group-3: Received ethanolic extract of stem bark of <em>Hugonia mystax</em> at a dose of 25mg/ml.</td>
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<tr>
<td>Group -4: Received ethanolic extract of stem bark of <em>Hugonia mystax</em> at a dose of 50mg/ml.</td>
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<tr>
<td>Group-5: Received ethanolic extract of stem bark of <em>Hugonia mystax</em> at a dose of 100mg/ml.</td>
</tr>
</tbody>
</table>

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. Observations were made for the time taken to paralysis and death of individual worms.

**RESULT AND DISCUSSION**

Preliminary Phytochemical analysis of ethanolic extract of stem bark of *Hugonia mystax* was presented in table 1. Preliminary phytochemical studies on *Hugonia mystax* showed the presence of carbohydrates, flavonoids, steroids, saponins, terpenoids and absence of alkaloids, proteins and amino acids.

<table>
<thead>
<tr>
<th>Phytoconstituents present in the ethanolic extract of stem bark of <em>Hugonia mystax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoconstituent</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Steroids</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
<tr>
<td>Terpenoids</td>
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<tr>
<td>Tannins</td>
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<tr>
<td>Alkaloids</td>
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### Table 2. Anthelmintic activity of ethanolic extract of stem bark *Hugonia mystax*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th><em>Pheretima posthuma</em></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paralysis time (minutes)</td>
<td>Death time (minutes)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Albendazole</td>
<td>10 mg/ml</td>
<td>28.55±1.16</td>
<td>58±1.03</td>
<td></td>
</tr>
<tr>
<td>Ethanol Extract of stem bark of <em>Hugonia mystax</em></td>
<td>25 mg/ml</td>
<td>55.15±1.17</td>
<td>126.2±1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>48.7±1.13</td>
<td>118.9±1.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/ml</td>
<td>35.1±1.05</td>
<td>66.4±0.82</td>
<td></td>
</tr>
</tbody>
</table>

All values represent Mean ± SD, n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant.

**Fig 1. Anthelmintic activity of ethanolic extract of stem bark *Hugonia mystax***

The anthelmintic activity of stem bark of *Hugonia mystax* was carried out on *Pheretima posthuma*. Different concentrations of the ethanolic extracts were used for the studies. The time taken for paralysis and death of earthworms were recorded in table 2. The data reveals that the ethanolic extract at the concentration of 25, 50 and 100 mg/ml showed death time 126.2, 118.9 & 66.4 min respectively. The effect increased with concentration of extracts on dose dependent manner. The extract caused paralysis followed by death of the worms at all dose levels. The anthelmintic activity of ethanolic extract of stem and bark of *Hugonia mystax* was compared to the standard drug Albendazole. Figure 2 showed graphical presentation of Anthelmintic activity of ethanolic extract of stem and bark *Hugonia mystax*. Phytochemical analysis of the crude extracts revealed the presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activities [17]. Chemically tannins are polyphenolic compounds [18]. Some synthetic phenolic anthelmintics (eg) niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [19]. 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 gastrointestinal tract of host animal [20] or glycoprotein on the cuticle of the parasite [21-22] and cause death.

**CONCLUSION**

The traditional claim of stem bark 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.
REFERENCES

17. Niezen JH, Wagborn GC, Charleston WAG. Growth and gastro intestinal nematode parasitism in lamps grazing either Lucerne (Medicago sativa) or sull (Hedysarum coronarium), which contains condensed tannins. J Agri Sci, 1995, 125, 281-289.