



## ANTHELMINTIC ACTIVITY OF METHANOLIC LEAF EXTRACT OF *SOPHORA INTERRUPTA*

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### ABSTRACT

Crude methanolic extracts of leaves of *Sophora interrupta* (Fabaceae) were evaluated for in vitro anthelmintic activity on the Indian adult earthworms *Pheritima posthuma*. Albendazole was used as a standard drug to compare the test results. The results were interpreted as time taken for paralysis and death of the earth worms. The study concluded that the methanolic leaf extract of *Sophora interrupta* showed significant Anthelmintic activity in a dose dependent inhibition of spontaneous motility of earthworms.

**Keywords:** *Sophora interrupta*, anthelmintic, leaf, methanolic, *Pheritima posthuma*

### INTRODUCTION

Gastro intestinal parasites create a serious threat to the production of live stock in developing nations<sup>1</sup>. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Helminthes parasite infections are global problems with severe social and economic repercussions in the third world countries<sup>2</sup>. The diseases affect the health status of a large fraction of human population as well as animals. Some type of dangerous helminthes infections like filariasis has only a few therapeutic modalities at present<sup>3</sup>. Helminthes infections are commonly found in community and being recognized as cause of much acute as well as cattle's use of herbs could be one of the major options to control these pathologies. The literature survey reveals that *Sophora interrupta* is used to treat various types of gastrointestinal problems. Therefore an attempt has been made to evaluate anthelmintic activity of leaves on adult earthworm *Pheritima posthuma*. *Sophora interrupta* belongs to the family fabaceae is commonly called as Edwardsia maderaspatana Wight, Pili Girgoli. There are approximately 219 species in genus *Sophora*. *Sophora interrupta* is available exclusively in Seshachalam hill ranges of Tirumala, India. This plant is woody perennial shrub with pinnate leaves, sub opposite leaflets broadly ovate and golden yellow flowers. It has multifarious medicinal properties<sup>4,5</sup>. The plant was investigated and was found out to possess abortifacient, anti-bacterial, anti-cholesterolemic, anti-inflammatory, anti-spasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic and tonic properties<sup>6</sup>.

### MATERIALS AND METHODS

#### Collection and Authentication of Plant

The leaves of *Sophora interrupta* were collected from surroundings of Seshachalam hill ranges of Tirumala, Tripathi, Andhra Pradesh, India during the month of August. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Associate professor, Department of Botany, S.V. University, Tripathi<sup>7</sup> and a copy has been preserved for the future reference at the herbarium

of the institute TRR College of Pharmacy. The leaves of *Sophora interrupta* were dried in the shade, milled into coarse powder by a mechanical grinder and stored in air tight closed container for further use.

#### Preparation of the Plant Extract

The air dried coarse powder of the leaves of *Sophora interrupta* was extracted with methanol using soxhlet's apparatus. The powdered material (2 kg) was defatted with petroleum ether (60-80°C) in a soxhlet extraction apparatus and marc was extracted with methanol (1000 mL) over night, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The crude extract was dissolved in distilled water to required concentrations and used for the experiments. The crude extracts were subjected to qualitative tests for the identification of various active constituents. From the dried extract, accurately 5 mg / ml, 10 mg / ml, 15 mg / ml, 20 mg / ml, 25 mg / ml, 30 mg / ml suspensions of methanolic extract of *Sophora interrupta* in normal saline was prepared.

#### Phytochemical Screening

Phytochemical screenings were performed using standard procedures as follows<sup>8,9</sup>.

#### Test for Reducing Sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

#### Test for Anthraquinones

The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

**Test for Terpenoids (Salkowski test)**

To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

**Test for Flavonoids**

A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

**Test for Saponins**

To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Test for Tannins**

About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride (FeCl<sub>3</sub>) was added and observed for brownish green or a blue-black coloration.

**Test for Alkaloids**

0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

**Test for Cardiac Glycosides (Keller-Killiani test)**

To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under laid with 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

**Animals**

*Pheretima posthuma* (Adult Indian earth worms) of about 5-7 cm long were used for the present study.

**Standard Drug used**

Albendazole suspension (micronized albendazole suspension in the concentration of 10 mg / ml) was used as the standard to compare the test results.

**Anthelmintic Activity**

*Pheretima posthuma* (Indian adult earth worms) of nearly equal size (6 cms ± 1) were selected randomly for the present study<sup>10-12</sup>. The worms were acclimatized to the laboratory conditions before experimentation. The earth worms were divided into four groups of six earth worms in each. Albendazole suspension in the concentration of 10 mg / ml served as a standard and poured into petri dishes. The test extract were prepared in the concentrations of 5 mg / ml, 10 mg / ml, 15 mg / ml, 20 mg / ml, 25 mg / ml, 30 mg / ml. Normal saline served as control. Six earth worms nearly equal size 6 cms ± 1 were taken for each concentration and placed in petri dishes at room temperature<sup>13</sup>. The time taken for complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each sample was calculated. The time taken for the worms to be become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates or induce movements in the earthworm, if alive<sup>14</sup>.

Table 1: Anthelmintic Activity of Methanolic Extract of *Sophora interrupta* leaves

Treatment	Concentration used (mg/ml)	Time taken for paralysis (min) X ± S.D	Time taken for death (min) X ± S.D
Control	---	---	---
Standard (Albendazole)	10 mg / ml	17 ± 1.571*	39 ± 1.932*
Methanolic extract of <i>Sophora interrupta</i>	5 mg / ml	76 ± 3.303*	99.33 ± 0.402*
	10 mg / ml	65.33 ± 2.883**	90.67 ± 3.921**
	15 mg / ml	56 ± 2.017**	85.00 ± 5.310**
	20 mg / ml	4.33 ± 1.498**	69.83 ± 2.496**
	25 mg / ml	48.33 ± 1.498**	51.67 ± 2.108**
	30 mg / ml	27.33 ± 2.060**	40.33 ± 0.9888***

All values are Mean ± SEM analyzed by one way ANOVA followed by Dunnett's test. \*p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

**RESULTS**

The earlier studies on preliminary phytochemical investigations of *Sophora interrupta* plant revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, flavonoids and poly phenolics. Methanolic extracts of *Sophora interrupta* were used to evaluate anthelmintic activity and showed the effect in a dose dependent manner. The mean ± SEM values (statistical analysis) were calculated for each extracts. The results of anthelmintic activity on earth worm *Pheretima posthuma* was given in Table 1, reveal that, the different concentration of the extracts has shown paralysis and death of earthworms and it was compared with albendazole as reference drug. Methanolic extract in the

concentration of 30 mg / ml has taken less time to cause paralysis, and little more time to cause death of earthworms as compared with reference drug.

**DISCUSSION**

Some of the traditionally used herbs have scientifically proved a potent anthelmintic activity by using suitable experimental models. The predominant effect of Albendazole on the worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. The

extract demonstrated paralysis as well as death of the worms at a time comparable to Albendazole especially at higher concentration of 30 mg / ml. Poly phenolic compounds shown anthelmintic activity; chemically tannins are poly phenolic compounds. Some synthetic phenolic anthelmintics, Eg: - Niclosamide, Oxytoclozanide and Bithionol are shown to interface with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that the active principle like tannins in the extract of *Sophora interrupta* produces similar effects. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastro intestinal tract of host animal or glycol protein on the parasite and cause death<sup>15</sup>. Further the extract can be tested on various other helminthes to ascertain the anthelmintic activity on a broader scale which is our future plan of research work.

### CONCLUSION

From the above result it is concluded that methanolic extracts of *Sophora interrupta* have a potent anthelmintic activity when compared with conventionally used drug. It is comparable with standard drug. Further studies using in vivo model are required to find out and to establish effectiveness and pharmacological rationale for the use of leaves as anthelmintic drug. Further studies to isolate active constituent from extracts to establish (s) mechanism of action are required.

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