

Research Article

Evaluation of In-Vitro Anthelmintic Activity of Ethanolic and Aqueous Extract of *Sesbania sesban* Leaves

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ABSTRACT

Aim: the aim of the present study was to evaluate the anthelmintic potential of *Sesbania sesban* leaves using *Pheretima posthuma* (earth worm) as test worms. **Method:** Varying concentrations of ethanolic extract of *S. sesban* leaves were taken (10, 20, 30, 40, 50mg/ml) were tested in bioassay which mainly involved the determination of certain vital parameters such as time of paralysis and time of death of the worms. Piperazine Citrate at varying concentrations (10, 20, 30, 40, 50mg/ml) is taken as the standard reference and normal saline is used as the control. **Result:** The result of the present study shows that the ethanolic extract of *Sesbania sesban* leaves showed significant anthelmintic activity by causing paralysis followed by the death of *Pheretima posthuma* worms but not as significant when compared to the standard reference Piperazine citrate. **Conclusions:** The use of the leaves of the plant *Sesbania sesban* as anthelmintic has been confirmed and further research should be done in order to isolate the active ingredients which are basically responsible for its anthelmintic activity.

Keywords: Anthelmintic activity, Piperazine citrate, *Pheretima posthuma*, *Sesbania sesban*.

INTRODUCTION

Sesbania sesban is a shrub or a short lived tree up to 8m tall with stem upto 12cm in diameter, usually pubescent, sometimes becomes glabrous. Leaves consist of a short petiole, 2-8 cm long; leaflets in 6-27 pairs¹⁴. It is widely distributed over the continents of Africa, Asia, and Australia, but Africa is presumed to be the center of diversity with subsequent distribution most likely by man. *Sesbania sesban* has a long history of use in India, primarily as manure, as a good source of protein for sheep and cattle. Also used as a live support for black pepper, grapes, betel vine and as a shade tree for coffee and turmeric¹⁵. In Africa *Sesbania sesban* roots and leaves are used to treat scorpion stings, boils and abscesses, also used to treat sore throat, gonorrhoea, syphilis, spasmodic fits in children and jaundice during pregnancy¹⁶. In other countries the leaves are used as tea and are considered to have antibiotic, anthelmintic, antitumor and contraceptive properties. Oil from the seeds is specially used in ayurvedic medicines and is reported to have bactericidal, cardiac depressant and hypoglycemic action. Parasitic disease cause severe morbidity by affecting population in endemic areas with major economic and social consequences¹. A number of medicinal plants have been used to treat parasitic infections in man and

animals^{2,5}. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. More than half the world suffers from various types of infections and majority of the cattle suffer from worm infections⁶. Intestinal infections with worms can be more easily treated than those infections occurring in other locations in the body, because the worms need to be killed by the drug and the drug need not be absorbed when given by oral route. However, increasing problems of development of resistance in helminthes^{7,8} against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. Therefore an attempt has been made to evaluate anthelmintic activity of leaves of *Sesbania sesban* on adult earth worm.

MATERIALS AND METHODS

Collection of plant

The fresh leaves of *Sesbania sesban* were collected in the month of May from the college campus in Valachil, Mangalore and were authenticated by Mr Gopikrishna asst. professor, Department of Pharmacognosy, Srinivas college of Pharmacy, Valachil, Mangalore.

Extraction of Plant Material

The leaves were shade dried and were ground to coarse powder. Powder was then extracted with ethanol which was further evaporated to dryness to obtain dry residue. Aqueous extract was also obtained by decoction method with fresh leaves powder by heating upto 1.5 hours

9.

Experimental Procedure

Ethanol and aqueous extracts from the leaves of *Sesbania sesban* were investigated for their anthelmintic activity against *Pheretima posthuma*. Various concentrations (10, 20, 30, 40, 50 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was used as standard reference and the distilled water as control. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings¹⁰⁻¹³. Because of easy availability, earthworms have been used widely for initial evaluation of anthelmintic compounds *in vitro*¹⁸⁻¹⁹. Indian adult earthworms collected from moist soil were washed with normal saline to remove all fecal matter and were used for anthelmintic study. The earthworms of 3-5 cm length were used for all the experimental groups. Sixteen groups of six earth worms were released into 10 ml of normal saline. Then Piperazine citrate, aqueous and ethanolic extracts of leaves of *Sesbania sesban* (10, 20, 30, 40, 50 mg/ml) in distilled water were added to their respective group. Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was confirmed when worms lost their motility when dipped in warm water

(50°C) followed with fading away of their body colours. All the values are expressed as mean \pm S.E.M for groups six earthworms each. This method was analyzed by one way ANOVA and compared by using Dunnett's comparison test.

RESULT AND DISCUSSION

Preliminary phytochemical screening of leaves of *Sesbania sesban* showed the presence of alkaloids, phyto-sterols, phenols, flavonoids, saponins and may be trace amounts of gums and fixed oils. It was found that ethanol and aqueous extracts exhibited anthelmintic activity in a dose dependent manner giving the shortest time of paralysis and death at 50mg/ml concentration. The ethanolic extract of *Sesbania sesban* caused paralysis in about 17-18 minutes and the time of death in 25-27 minutes while the aqueous extract caused paralysis between 9 -13 minutes respectively against earthworm *Pheretima posthuma*. The reference drug Piperazine Citrate showed paralysis between 5-14 mins and death occurred between 14-19 minutes respectively. Piperazine Citrate exhibits anthelmintic activity by blocking the glucose uptake and depletion of glycogen stores in the parasite. The aqueous extract of *Sesbania sesban* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50mg/ml in shorter time when compared to the ethanolic extract of *Sesbania sesban*. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds. It is possible that tannins contained in the extracts of *Sesbania sesban* produce similar effects¹⁷. Reported anthelmintic effect of tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death¹⁸. Further studies are under process to identify the possible phytoconstituents responsible for anthelmintic activity.

Table 1: Anthelmintic activity leaf extracts of *Sesbania sesban*

S.No	Treatment	Dose(mg/ml)	Time for paralysis (mins)	Time for death (mins)
1	Control	-----	-----	-----
2	Ethanol	10	19.50 \pm 0.3416	27.67 \pm 0.2108
3	Ethanol	20	19.50 \pm 0.3416 ^{ns}	27.83 \pm 0.1667
4	Ethanol	30	18.83 \pm 0.3073	27.67 \pm 0.2108*
5	Ethanol	40	18.17 \pm 0.1667*	26.67 \pm 0.2108**
6	Ethanol	50	17.83 \pm 0.3073**	25.67 \pm 0.2108**
7	Aqueous	10	12.50 \pm 0.2236***	22.83 \pm 0.1667***
8	Aqueous	20	12.00 \pm 0.000***	22.33 \pm 0.2108***
10	Aqueous	30	9.000 \pm 0.3651***	22.00 \pm 0.0***
11	Aqueous	40	8.000 \pm 0.3651***	21.33 \pm 0.2108***
12	Aqueous	50	6.500 \pm 0.2236***	21.17 \pm 0.1667***
13	Standard	10	5.333 \pm 0.2108***	18.67 \pm 0.2108***
14	Standard	20	5.000 \pm 0.3651***	18.17 \pm 0.1667***
15	Standard	30	4.500 \pm 0.3416***	17.50 \pm 0.2236***
16	Standard	40	3.500 \pm 0.2236***	15.33 \pm 0.4216***
17	Standard	50	3.000 \pm 0.2582***	13.17 \pm 0.4014***

Each value represents mean \pm S.E (n=6) and was analysed by ANOVA Tukey-Kramer multiple comparison test.*P<0.05, **P<0.01, ***P<0.001.

CONCLUSION

In conclusion, the traditional use of the leaves of *Sesbania sesban* as anthelmintic has been confirmed using the different extracts and showed good anthelmintic activity. Further it would be of great interest for detailed study of the phytoconstituents which are responsible for the anthelmintic activity and the mechanism of action.

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