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In Vitro Anthelmintic Activity of Methanolic Extract of *Senna tora* L. Seeds

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Abstract
Parasitic infection including Helminthiasis is a critical problem in the tropical region and gastro-intestinal helminthes are becoming resistant to currently available drugs. Hence there is an increasing demand towards natural anthelmintics. The aim of the work was to investigate the anthelmintic activity of *Senna tora* seeds. Methanol extract from the seeds of *Senna tora* were investigated for their anthelmintic activity against Earthworms and Housefly worms at different concentrations (25, 50 and 100 mg/ml) which involved the determination of time of paralysis and time of death of the worms. Albendazole was included as reference standard and distilled water as control. The study found that there is significant difference between *Senna tora* seeds, Albendazole and distilled water. The seeds extract was able to both paralyzed and extirpated the worms unlike the standard drug that only cause paralysis of the worms and hence the seeds of *S. tora* possess potent anthelmintic activity.

Keywords: Helminthiasis, *Senna tora* L., Earthworms, Housefly worms, Anthelmintics

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Introduction

Senna tora Linn. (Family; Fabaceae) is a small shrub that grows in warm moist soil throughout the tropical parts of Asian and African countries. It is commonly known as "Tafasa (Hausa)" in Nigeria, "Coffee pod" in Korea, and "Chakramarda" in India. The seeds contain anthraquinones, Phenolic glycosides, Brassinosteroids, Monoglycerides Rhein, Aloe emodin, Rubrofusarin and Chrysophanic acid (Jang *et al*., 2007). Traditionally, the plant have been widely used in India, China and Korea against different diseases such as being hepatoprotective (Tiwari *et al.*, 2011), anti-inflammatory (Jayasutha and Nithila, 2011, hypolipidemic (Patil *et al*., 2004), hypotensive (Hyun *et al*., 2009), purgative (Maity & Dinda, 2003), antidiabetic (Chaurasia *et al.*, 2011), antiulcer (Gulia and Choudhary, 2011), antifungal (Mukharjee *et al*., 1998), and Nitric Oxide scavenging activities (Rejiya *et al*., 2009). Due to the moist quality, sweet flavor and cold property of the seeds, they act on large intestine channels, improve vision and ease the bowels by clearing the heat and nourishing the large intestine. Externally, it's applied as a germicide and antiparasitidal, eases skin itch when mixed with lime juice. Dried seeds contain 20-24% protein and are given as a protein rich feed to livestock and birds (Jain and Patil, 2010). They also yield tannins, yellow, blue and red colored dyes, 7.5% gum which is a good suspending and binding agent and also have gelling properties (The Ayurvedic Pharmacopeia of India, 2001; Jain and Patil, 2010).

Helminthiasis is a parasitic disease of humans and animals in which a part of the body is infected with parasitic worms such as pinworms, roundworms, or tapeworms. These parasitic worms are vectored

through air, food and water (Murugamani *et al*., 2012). Intestinal helminths infections are among the most common and neglected public health problems in many developing tropical countries. Worldwide, more than 3.5 billion people are infected with intestinal worms (Pullan & Booker, 2012), and an estimated 4.5 billion individuals are at risk of soil-transmitted helminth infection (Jourdan *et al.*, 2018). Anthelmintic drugs are used to destroy or cause the expulsion of such parasitic intestinal worms, one of the most common infections in humans and cattle. Albendazole is the first drug of choice for the treatment of worm infections. The gastro-intestinal helminths are becoming resistant to currently available anthelmintic drugs and to overcome the resistance, plant derived drugs may serve as model to develop more effective and less toxic medicines. Hence there is an increased demand towards natural anthelmintics (Rashedul *et al*., 2012).

The present study involves the investigation of *in vitro* anthelmintic activity of methanolic extract of *S. tora* seeds against housefly worms and earthworms using distilled water as control with a view to justify the use of the plant in the treatment of helminths and since there is no similar study carried out in relation to that in Gombe State, Nigeria this research will therefore fill the gap left.

Materials and Methods

Plant material and preparation of extract

The fresh seeds of *S. tora* were collected in the month of October, 2017 from the wild, authenticated by the university herbarium and given a voucher number GSU 011. Shade-dried seeds were ground to coarse powder; powder was first defatted by soaking in petroleum ether with regular shaking for 48 hours and then extracted with methanol using Soxhlet apparatus. The extract was then further evaporated to dryness using rotary evaporator to obtain the methanolic extract.

Experimental Animals

The assay was performed on adult earthworms (*Eisenia fetida)* owing to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Khandelwal, 2000; Ajaiyeoba *et al*., 2001) and Housefly worms owing to their resemblance with pinworms in human infections as reported by Murugamani *et al*., (2012)**.** Availability of earthworms prompts their extensive use for preliminary *in vitro* evaluation of anthelmintic compounds (Dash *et al*., 2002). Housefly worms are used for anthelmintic activity study because of their prominence and adaptability to laboratory conditions (Murugamani *et al*., 2012)**.**

Earthworms

Adult earthworms (*Eisenia fetida)* of 6-8cm in length and 0.1-0.2 cm in width were collected from moist soil and washed with distilled water to remove all dirts.

Housefly worms

Houseflies, *Phaenicia sericata* (Green bottle fly) were collected in a sterile hand net from a fruit seller in Gombe State University commercial area. The flies were identified and authenticated by an expert in the Department Biological Sciences, Gombe State University.

Five matured female houseflies were placed in the plastic jar containing substrate and covered with a sieve material to prevent other flies from entering. Moisture range of 60-75% and temperature range of 25-30ºC were maintained as housefly worms are inactive and develop poorly at temperatures below 20ºC and less than 50% moisture. After 24 hours, eggs which were very small and white in color were laid and the dead flies removed. The substrate containing the eggs was further kept for 48hrs at room temperature in a dark place, resulting in the formation of worms with an average count of 100 worms of uniform size, weight (0.01 g) and age (48hrs). The worms were collected using the method of Sogbesan *et al*. (2006) (screening method) where the substrate was spread in a thin layer on a sieve (2 mm) placed over a clean basin under sunlight; the worms tried to escape the sunlight by passing through the screen and drop into the basin. The collected worms were washed with distilled water to remove all dirts.

Drugs and Chemicals

Albendazole was used as positive control; Amoxicillin and Chloramphenicol were used as antibiotics against the growth of other microorganisms. All these drugs were purchased from an accredited pharmaceutical store. Also Methanol was used as a solvent, powdered seed of *S. tora* was soaked in Petroleum ether and Dimethyl sulfoxide (DMSO) was used as a solvent (Ajaiyeoba *et al*., 2001).

Anthelmintic Activity

The anthelmintic assay was carried out according to the method of Ajaiyeoba *et al*. (2001). All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Five groups of earthworms with each group containing five worms of approximately equal size (6-8 cm) were released in to solutions of three different concentrations (25, 50 and 100 mg/ml) of the methanolic extract of the plant material in petri dishes. Albendazole (400 mg/ml) was used as positive control and distilled water as negative control. Time of paralysis and time of death of the worms were recorded. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded when the worms neither moved when shaken vigorously nor when dipped in warm water followed by fading away of their body colors (Ajaiyeoba *et al*., 2001). The same experiment was done for the housefly worms and the time of paralysis and death also recorded.

Statistical Analysis

The results were expressed as Mean values ± S.E.M. (standard error of mean). Statistical comparison was carried out by one way Analysis of Variance (ANOVA) using SPSS 16.0 and the significance difference between the means were separated using L.S.D (Least Significant Difference). The results were considered to be statistically significant when $P < 0.05$.

Results

Anthelmintic Activity in Earthworms

The methanolic extract of *S. tora* seeds at different concentrations demonstrate immediate death of the worms without paralysis in a less time compared to the reference standard Albendazole (400 mg/ml) especially at higher concentration of 100 mg/ml while the control survived for an average time of 23hrs (Table 1).

Anthelmintic Activity in Housefly worms

The result of the anthelmintic activity of *S. tora* seeds methanolic extract at different concentrations and Albendazole (400 mg/ml), based on time of paralysis and time of death was shown in Table 2. The time taken for both paralysis and death in all the three concentrations of the extract was less compared to that of the standard drug Albendazole (400 mg/ml) while the ones in control lived for an average time of 49hrs.

Table 1: Anthelmintic Activity of methanolic extract of *S. tora* seeds on Earthworms

Test drugs	Concentrations (mg/ml)	Time	
		Paralysis	Death
S. tora extract	25	-	0.31±0.02
	50	-	0.25±0.01
	100	-	0.15±0.03
Albendazole	400	10.00±0.94	89.00±2.08
Control	-	1,240.00±71.27	1,380.00±91.65

Table 2: Anthelmintic Activity of methanolic extract of *S. tora* seeds on Housefly worms

Test drugs	Concentrations (mg/ml)	Time	
		Paralysis	Death
S. tora extract	25	18.00±0.24	51.33±0.45
	50	12.17±0.09	46.04±0.27
	100	8.78±0.16	40.22±0.09
Albendazole	400	8.71±0.18	1,830.00±17.32
Control	-	2,790.00±51.96	2,940.00±34.64

Discussion

In this anthelmintic assay, methanolic extract of *S. tora* seeds demonstrated paralysis as well as death of worms. As shown in Tables 1 and 2, the extract exhibited anthelmintic activity in dose dependent manner giving the shortest time of paralysis and death with100 mg/ml concentration. Higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all the worms. Evaluation of the anthelmintic activity was compared with reference standard drug Albendazole (400mg/ml) and the methanolic extract of *S. tora* seeds was found to be more effective in causing paralysis and death of all the two types of worms. This is similar to the work of Gaikwad *et al*. (2017) who found out that in an experiment to evaluate the anthelmintic activity of *S. tora* seeds and seeds cover using ethyl acetate, acetone, water and methanol extracts along with Albendazole as reference standard, the activity of the methanolic extract happened to be more effective than the rest by having less time of paralysis and time of death compared to all including the standard drug.

The anthelmintic activities of Albendazole and the different concentrations of the methanolic extract of the *S. tora* seeds in housefly worms and earthworms were performed under laboratory conditions and the result of the anthelmintic activities in housefly worms compared with that in earthworms showed that the housefly worms had taken more time for paralysis and death. It was observed that the earthworms do not adapt easily to the laboratory conditions unlike the housefly worms that were grown in the laboratory. This is in line with the work of Murugamani *et al*. (2012) who reported that the evaluation of anthelmintic activity of any drug when carried out in the laboratory conditions by using isolated worms from nature cannot be adaptable with artificial laboratory conditions like when the worms are grown in the lab and hence the housefly worms had taken more time for paralysis and death in spite of smaller size and lesser weight of the worms compared to the earthworms.

The earthworms used for this study were very sensitive to the methanolic extract of *S. tora* at all the concentrations and thus causing immediate and direct death of the worms at a very small time without even paralysis. This is in contrary with the findings of Gaikwad *et al*. (2017) who reported that the methanolic seed extract of *S. tora* demonstrated paralysis as well as death of worms in a less time as compared to Albendazole, ethyl acetate and acetone extracts especially at higher concentration of 100 mg/ml. This may be due to edaphic changes and changes in climatic conditions of the places where the worms were taken from.

Conclusion

The vermicidal activity of the methanol extract of *S. tora* seeds as described herein against earthworms and housefly worms shows that it has an anthelmintic activity even better than that of the standard drug Albendazole because it was able to both paralyzed and extirpate the worms unlike the standard drug that only caused paralysis of the worms. Hence the seeds of *S. tora* possess potent anthelmintic activity. There is need for further clinical studies to know the mode of action and dosage.

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