Original Article

In-vitro Anthelmintic Activity of Saba Florida (Benth) Extracts Against Nigerian Adult Earth Worm (*Terrestris lumbricoides)*

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ABSTRACT

Introduction: *Saba florida* (*Benth*) *Apocynaceae* is one of the African food/medicinal plants that has been applied traditionally for the management of many ill health conditions.
Objectives: As part of efforts to authenticate the oral claims scientifically the plant's leaf and stem bark extracts were evaluated for anthelmintic activity.
Methods: Standard procedures were used in the determination of anthelmintic activity study. Nigeria adult earthworms (*Terrestris lumbricoides*) were used for the study. Albendazole was used as standard. All the earthworms were washed in distilled water before they were released into a 20ml of respective formulations including the control, standard and extracts (10, 20, 50, and 100mg/ml).
Results: All the investigational extracts showed anthelmintic activity at the dose of 20mg/ml. Time taken for paralysis and death to occur were recorded for each sample. The aqueous extract showed better activity against the worms than the chloroform extract. The extracts' anthelmintic activity was dose-dependent and comparable to the standard drug used.
Conclusion: From this study herbal drug and synthetic ones are equally effective in helminth infestations, but aqueous extract of the leaf of *Saba florida* showed higher anthelmintic activity potential than the chloroform extracts.

Keywords: Albendazole, *Saba florida*, *Terrestris lumbricoides*, Anthelmintic activity.

INTRODUCTION

Worm infections or parasitic worms affect several million people across the globe. In developing countries they are the major threat to public health, key contributing factor to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. In the tropics there exist simultaneous infections with more than one type of helminth[1,2].

Parasitic diseases causing severe morbidity include lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences[1].

Helminthosis plays a crucial role in the small ruminant production, leading to enormous economic losses, particularly in areas where extensive grazing is practiced.

Helminthes may be limited solely to the intestinal lumen or may involve a complex process with the migration of the adult or immature worm through the body before localization in a particular tissue. Complicating scientists' understanding of the host-parasite relationship and the role of chemotherapy in helminth-induced infections are the complex life cycle of many of these organisms, whereas some helmets have a simple cycle of egg deposition and development of the egg to produce a mature worm[3].

Helminths can be divided into the following major groups: *Cestodes* (Flatworms) *nematodes* (roundworm) *trematodes* (flukes) and, less frequently, *Acanthocephala* (thorny-headed worms)[4,5]. As per World Health Organization (WHO) only synthetic drugs are frequently used in the treatment of helminth infections in human beings, but these synthetic drugs are out of reach of millions of people and have a lot of side effects[6].

Development of resistance to most commercially available anthelmintics became a severe problem worldwide. In view of this, attempts are being made the world over to study the anthelmintic activity of herbal drugs. The limited availability and affordability of pharmaceutical medicines mean that the world population depends to a greater extent on traditional medical remedies, and some 20,000 species of higher plants are used medicinally throughout the world. Anthelmintics from the natural sources may play a key role in the treatment of these parasitic infections and can avoid resistance to synthetic agents[6].

Saba florida (Benth) Apocynaceae, is one of the African medicinal plants which has not been fully studied scientifically. The plant is found often on stream-banks, commonly through the region from Senegal to west Cameroon and across Africa to Sudan, Zansibar and Tanzanika. The plant is found in Ibaji and other parts of Kogi State, Nigeria. Bark and root decoctions are taken during drought in Tanzania as an aphrodisiac, a root decoction as a vermifuge and leaf decoction as a laxative[7]. The root together with other substances is given in Nigeria as a remedy for gonorrhea [8] and in Tanzania is also used as a snake bite remedy. The fruit has an edible pleasantly acid pulp. It is much relished by nearly all races wherever the plant occurs, and by monkeys[8]. In Zanzibar, a fruit drink is taken not only as pleasant drink but to cure pimples. The leaves are eaten as an antidote against vomiting and the bark decoction also are administered for diarrhea and food poison[9]. We have also reported the antioxidant properties of *Saba florida in vitro*. It is relatively non-toxic[9]. *Saba florida* parts were found to contain vitamins A and E, fatty acids and other nutrients[10].

From literature reviews it appears there are no works carried out on *Saba florida* for anthelmintic activity. The sole objective of this study is to evaluate anthelmintic potential of extracts of leaf and stem of *Saba florida* to support or disprove scientific the oral claims as to its efficacy as an anthelmintic agent.

MATERIALS AND METHODS

Drug and chemicals: Albendazole was purchased from Cuzark pharmacy Limited, Anyigba, Nigeria. Chloroform used was of analytical grade from BDH.

Tween80® (poly oxyethylene-sorbitan monooleate; polysorbate 80), distilled water.

Plant material/methods

The plant materials used for this study are powdered leaf and stem bark of *Saba florida (Benth*). The plant material was collected from Odogwu in Ibaji Local Government Area, Kogi State, Nigeria during dry season. Dirt was removed from the plant parts by rinsing in clean water. The leaf and stem bark were air-dried for three (3) weeks and pulverized, using motorized blender into powder and sieved. The plant was identified by a botanist-Mr Baba Ekwuno in the Botany unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *Saba florida* (Benth) Bullock.

Preparation of plant extracts

The fine plant material was subjected to aqueous and chloroform extraction.

Aqueous extraction

Portions of the powdered leaves (177g) and stem bark (283g) were weighed into 2000ml beakers and 1000ml, 1500ml of distilled water was added to the powdered leaf and stem bark respectively and left for 72 hours. The mixtures were filtered and evaporated to dryness at 60^0c in a water bath and the residue (extract) was used for the anthelmintic studies.

Chloroform extraction

Portions of powdered leaves (115g) and stem bark (281g) were weighed into 2000ml beakers; 500ml and 1000ml of chloroform (99.4%) were added respectively and left for 72 hours. The mixtures were filtered and evaporated to dryness at 45^0C in a water bath and the extract was used for anthelmintic activity study.

The standard used for the activity study

Albendazole (20mg/ml in distilled water) was used a reference drug while distilled water was used as negative control.

Experimental animal (Nigerian adult earthworm)

Nigerian earthworms-*Terrestris lumbricoides*, were used for the anthelmintic activity study of *Saba florida* extracts. The earthworms were obtained from the stream banks of Inachalo, Idah, Kogi State, Nigeria. Worms were authenticated by Mr. Okpanachi Marthins, a Zoologist in the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria. The worms were washed with distilled water to remove all the fecal matter and waste surrounding their body. The worms (*Terrestris lumbricoides*) 19-22cm in length, 1-2cm in width and 1.2-2.1g in weight were used for all experimental protocols. The earth worms resemble the intestinal parasites, both anatomically and physiologically and as such were utilized for anthelmintic activity investigation[11,12.]

Department of Biochemistry, Kogi State University guidelines for handling experimental animals was followed and approval for the use of Adult worm for the experiment was given by the Head of the Unit.

Preparation of test samples

Aqueous extract, test sample: The leaf and stem bark samples for *in vitro* study were prepared by dissolving and suspending (0.2, 0.4, 1 and 2g) of each aqueous leaf and stem bark extract in 20ml of distilled water, at different concentrations ranging from 10, 20, 50 and 100mg/ml.

Chloroform extract, test sample: Samples for *in vitro* studies were prepared by dissolving and suspending (0.2, 0.4 ,1 and 2g) of each chloroform leaf and stem bark extract in 20ml of 20% v/v tween 80 in distilled water, at different concentrations ranging from 10, 20, 50 and 100mg/ml.

Anthelmintic investigation

The anthelmintic bioassay was carried out following the method described by Ajayieoba *et al*. with minor modifications[13]. The assay was carried out in vitro utilizing Nigerian adult earthworm (*Terrestris lumbricoides*) owing to its anatomical and physiological resemblance with an intestinal roundworm parasite of human beings for preliminary investigation of anthelmintic activity[14-16]. The 20ml formulation containing different concentrations of aqueous extracts (10, 20, 50 and 100mg/ml in distilled water) and 20ml formulations containing four different concentrations of each chloroform extract (10, 20 50 and 100mg/ml in 20% v/v tween 80 in distilled water) was prepared and four worms (same type) were placed in them a transparent container. Time for paralysis (P) was noted when no movement of any sort could be observed except the worms were shaken vigorously. The time of death (D) of worms was concluded and recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in distilled water. Albendazole (20mg/ml) was used as positive control while distilled water was used as negative control.

Statistical Analysis

All results are expressed as mean $\pm$ S.E.M from four observations.

RESULTS

The aqueous leaf and stem extracts of *Saba florida* containing 10, 20, 50, and 100mg/ml produced dose-dependent paralysis ranging from loss of movement to loss of response to external stimuli, which gradually led to death. The leaf extract was more potent than the stem extract at the highest dose of 100mg/ml administered. Death time was 7.70$\pm$0.85 and 76$\pm$1.27 minutes respectively (Table 1).

Table 2 indicates the effect of the chloroform extracts on the adult earthworm. The extracts produced dose-dependent effects. The leaf extract is more potent than the stem bark extract and both produced activity which is comparable to the reference standard.

DISCUSSION

Nigerian adult earth worm, *Terrestris lumbricoides* is among the most important soil invertebrates that promote soil fertility. They achieve this through feeding and burrowing activities that break down organic matter and release nutrients and improve aeration, drainage and aggregation of soil. They have anatomical and physiological resemblance with other helmints[17].

The assay of biological activity, chloroform and aqueous extracts were used to investigate anthelmintic activity and showed a dose-dependent activity.

In table 1, the result of anthelmintic activity of the aqueous extract on the earthworm *Terrestris lumbricoides* is given and reveals that the different doses utilized to indicate paralysis and death of earthworms and it was comparable to the reference standard-Albendazole used.

Albendazole like any other anthelmintics such as piperazine cause hyperpolarization of worms' muscle by GABA agnostic action opening Cl- channels that cause relaxation and depresses responsiveness of contractile action of acetylcholine by increasing chloride ion

conductance of worm muscle membrane produced hyperpolarization and reduced excitability that could lead to muscle relaxation and flaccid paralysis[18].

The aqueous and the chloroform extracts did not just cause paralysis in, the worms, but the paralysis progressed to death at 20mg/ml to 100mg/ml.

The time difference between paralysis and death is shorter in the extract treatment compared to the drug (standard) used (Table 1&2).

The leaf extract has earlier been reported to have trypanocidal activities[19]. It is not surprising, therefore the anthelmintic activities demonstrated by the extracts. The antioxidant activity of the plant has been correlated with the presence of flavonoids, tannins and other secondary metabolites[9].

Tannins are shown to produce anthelmintic activities. Chemically they are polyphenolic compounds[20]. Possible mechanism of anthelmintic effect observed in the extracts could be due to the presence of tannins and terpenoids. These can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death.

In addition, tannins or their metabolites have a direct effect on the viability of the pre-parasitic stages of helminthes and other phytochemicals may be responsible for the anthelmintic effects[21]. Saponins mainly act by parallel irritation of mucus membranes that leads to parasite death[22].

CONCLUSION

The results of this investigation lend scientific support to the oral claims on the efficacy of *Saba florida* as anthelmintic agent. From the results, *Saba florida* as an anthelmintic agent has been confirmed as it demonstrated activity against the worms used in this present study. Further studies using *in vivo* model are required to find out and establish the effectiveness and pharmacological rationale for the use of leaf and stem of *Saba florida* as anthelmintic drug. In addition, isolation of the active principles responsible for the observed activity is needed and a goal to be pursued. From the afore mentioned pharmacological effects, it may be concluded that aqueous and chloroform extracts of *Saba florida* have anthelmintic activity and a potential drug candidate for elimination of parasitic worms.

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Conflict of interest statement

We all declare that we have no conflict of interest in this work.

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Table 1. Anthelmintic activity of aqueous leaf and stem bark extracts of *Saba florida*

Test substance	Concentration (mg/ml)	Paralysis Time (min.)	Death time (min.)	Time between paralysis and death (min)
Control (distilled water)	-	-	-	-
Standard (Albendazole)	20	142.21±12.48	173.20±2.30	30.99
Leaf extract	10	-	-	-
	20	55.61±10.79	188.29±8.72	132.67
	50	17.11±0.70	31.70±5.55	14.59
	100	5.05±0.25	7.70±0.85	2.65
Stem extract	10	-	-	-
	20	105.14±1.42	125.20±1.57	20.20
	50	75.94±5.96	88.69±2.89	17.75
	100	47.30±3.13	76.36±1.27	29.06

Results are expressed as mean ± S.E.M from four observations

Table 2. Effect of chloroform leaf and stem bark extracts of *Saba florida* on adult earthworm

Treatment	Concentration (mg/ml)	Paralysis time (min.)	Death time (min.)	Time between paralysis and death (min.)
Control (distilled water)	-	-	-	-
Standard (Albendazole)	20	142.21±12.48	173.20±2.30	30.99
Leaf extract	10	-	-	-
	20	143.75±2.69	151.00±5.52	7.25
	50	110.25±3.39	118.00±2.45	7.75
	100	86.25±1.50	98.50±0.74	12.25
Stem extract	10	-	-	-
	20	179.50±2.35	216.00±4.06	36.50
	50	150.25±1.50	165.25±2.45	15.00
	100	113.25±2.18	128.75±4.50	15.50

Results are expressed as mean ± S.E.M from four observations

Figure 1. *Saba florida* leaf and fruit

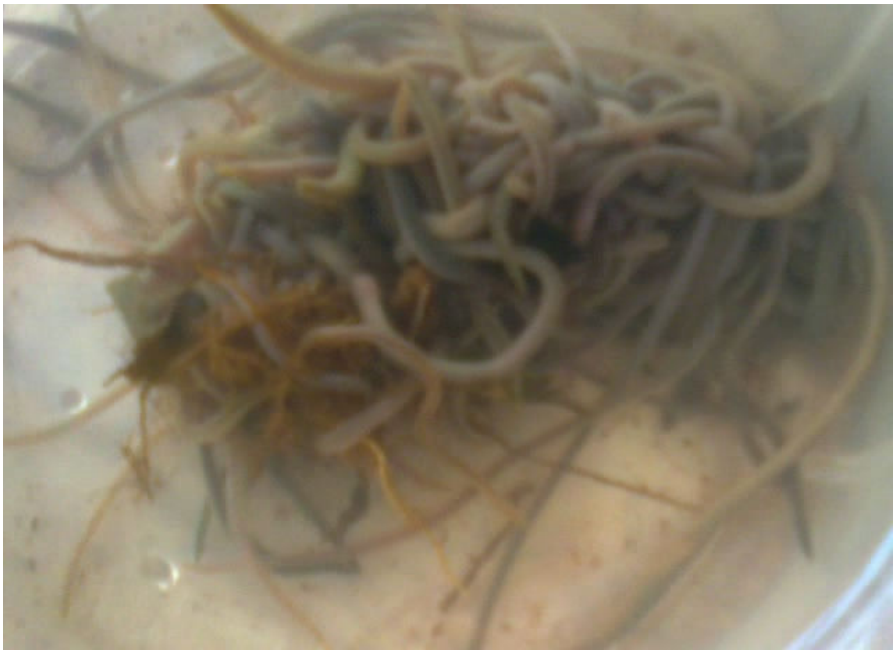

Figure 2. Nigerian adult earth worm (*Terrestris lumbricoides*)

Figure 3. Isolated worm from the mud