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Anthelmintic activity of *Securidaca longepedunculata* (Family: Polygalaceae) root extract in mice, *in vitro* and *in vivo*

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ABSTRACT

Objective: To elucidate the pharmacological bases of oral administration of *Securidaca longepedunculata* (*S. longepedunculata*) root extract as an anthelmintic in folkloric medicine. **Methods:** Albino mice were infected with infective third (L3) larval stage of *Heligmosomoides polygyrus* (*H. polygyrus*) by esophageal intubation. Following establishment of the adult worms in the intestine, the mice were treated with 0–2 000 mg/kg body weight (bw) of methanolic root extract of *S. longepedunculata* and 100 mg/kg bw of pyrantel embonate, the reference drug *in vivo*. Bioactivity and larvicidal effects of the extract were tested by exposing brine shrimps (*Artemia salina*) to 0.00–1.00 mg/mL and the L3 stage of *Heligmosomoides contortus* (*H. contortus*) and *H. polygyrus* to 0.00–2.50 mg/mL of the extract *in vitro*. **Results:** The percentage yield of the extract was 7.13% w/w dry matter. The brine shrimps toxicity bioassay resulted in an LC_{50} of 74.18 μg/mL. The extract had a significant, dose-dependent larvicidal effect on the L3 stage of *H. contortus* and *H. polygyrus* with the terminal effect of 75% and 70% at the highest exposure concentrations, respectively. The extract however, did not affect the number of worm eggs per gram (epg) of fecal materials ($P<0.05$) and total worm burden (twb) of adult *H. polygyrus* in infected mice. Treatment with pyrantel embonate significant reduced both the fecal egg count and twb to 0 compared to the untreated control ($P<0.05$). **Conclusions:** These results indicate that *S. longepedunculata* root extract contains potent bioactive compounds and has larvicidal effect on L3 stage of *H. contortus* and *H. polygyrus*, substantiating its use as anthelmintic in alternative medicine.

1. Introduction

For decades, extracts from plants have been used in alternative medicine for the treatment of helminthe infections and they still constitute part of therapy in today's traditional medical practice particularly in the tropics[1]. *Securidaca longepedunculata* (*S. longepedunculata*, Family: Polygalaceae) is increasingly popular in the Africa and other Tropical countries because of a wide variety of bioactive compounds that have found significant therapeutic applications in folkloric medicine. It is well-known and used in both western and southern part of Africa for different purposes including pest control and in the treatment of widely differing ailments. This plant is composed of several compounds including alkaloids, phenols, xanthones, flavonoids, terpenoids, anthraquinones, and saponin[2-4]. In agriculture, preparations of the root including root powder are used as pesticide in stored farm products such as grains with little environmental contamination that is often associated with synthetic pesticides[5-8]. Extracts from this shrub have shown activity against a variety of micro-organisms including bacteria[4,9,10], fungi[9], viruses[9],

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and protozoa[9]. There are suggestions that extracts of *S. longepedunculata* have prospects in the treatment of malaria, trypanosomiasis and inability to have, sustain or retain erection[3,9,11,12]. Root extract of this plant have shown remarkable benefits in analgesia[13], as an antioxidant[14], anti-inflammatory[13,14], and anti-depressant[14]. It has been used in the treatment of venereal and respiratory diseases in African traditional medicine[15] and as a teanifuge as well as vermifuge.

Helminthe parasites pose a serious health concern to humans and animals causing diseases and reduction in food animal production[16]. Infection by gastro-intestinal (GIT) nematodes constitute a major disease of economic importance that are often controlled by synthetic anthelmintic drugs predisposing these animals to be less sensitive to drug treatment and subsequently result in environmental contamination. With the increasing incidence of drug resistance and high cost of existing pharmacological agents, the search for new drugs from the medicinal plants is imperative[16,17]. Anthelmintic agents are a heterogeneous group of compounds that may either be synthetic or natural products that act against helminthe parasites[18]. A natural source of anthelmintic is in the diverse plant community and constitutes key component/ingredient in the treatment of helminthe infections in alternate medicine.

In the Nsukka local community of Nigeria, preparations from the root in combination with other substances are used as teanifuge and vermifuge. However, the rationale behind the use of the root extract of *S. longepedunculata* as an anthelmintic is not well-known let alone the specific bioactive compound responsible for this property. Because traditional medical therapy normally involves a combination of several plant extracts and sometimes a number of different plant sources, it is necessary to identify the specific plant source of the active agent as an early step in the isolation of the actual therapeutic substance(s). The objective of this study is to elucidate the pharmacological bases of using *S. longepedunculata* root extract as an anthelmintic in traditional African medicine. Using a laboratory mice/GIT nematode *Heligmosomoides polygyrus* (*H. polygyrus*) infection model[19,20], we hypothesized that the anthelmintic effect of *S. longepedunculata* is attributive to the root extract. Because, a potential anthelmintic agent ought to have a broad spectrum of activity on the target parasites including the different life stages; we therefore tested the anthelmintic effect of *S. longepedunculata* on the larvae of *Heligmosomoides contortus* (*H. contortus*) and *H. polygyrus in vitro* as well as on the egg and adult stage of *H. polygyrus in vivo*. Biological activity of the crude extract was initially tested using brine shrimps (*Artemia salina*) prior to the actual experiment.

2. Materials and methods

The experimental procedures were subjected to and were approved by the ethics committee of the University of Nigeria, Nsukka (UNN) in consonance with the guide to the care and use of laboratory animals in research and teaching in the institution. All the solvents and chemicals were of standard and analytic grade and the experimental solutions were freshly prepared.

2.1. Plant and extraction

The root system of *S. longepedunculata* was selected based on ethno-pharmacological information on its use as an anthelmintic. The plant material was collected from Adada Village in Nsukka Local Government Area (LGA) of Enugu State and identified by Mr A. O. Ozioko, a plant taxonomist in the Department of Botany, University of Nigeria Nsukka. Freshly collected root materials (60 g) were chopped into small bits and sun-dried for 5 d. It was then pulverized using electrically operated hammer mill and transferred into a large bottle with tightly-fitted lid containing 450 mL of 70% aqueous methanol. The bottle was allowed to stand for 24 h at room temperature (26 ℃) with intermittent shaking and subsequently filtered into a beaker. The solution was evaporated in the oven to dryness at 40 ℃ to give a yellowish powder dry matter.

2.2. Experimental animals

2.2.1. Mice

Albino Wistar mice (n = 30) of both sexes (approximately 20.15 g) were purchased from the laboratory animal's house of Mrs. G. Nwachukwu of the faculty of Veterinary Medicine, UNN. They were housed in clean aluminum (Al) cages and fed *ad libitum* with pelleted standard laboratory animal feed (Eagle brand quality feed). The mice also had free access to portable drinking water and were maintained in accordance with the stipulations of the Care and Use of Laboratory Animals in research of the University. They were acclimated for two week prior to experimentation and no mortality was recorded.

2.2.2. Helminthe parasites

Infective third larval (L3) stage of *H. contortus*, *H. polygyrus* as well as the adult worms of *H. polygyrus* were cultured from the worm eggs which were generously provided by Dr. B.B. Fakae of the Department of Veterinary Parasitology and Entomology, UNN. The parasite was cultured from the egg to L3 stage in petri dishes containing wet filter papers. Briefly, egg-containing fecal materials were macerated in the wet filter papers and incubated till they hatch into the first larval (L1) stage. The L1 metamorphosed into the second larval (L2)

and infective L3 stages, accordingly.

2.2.3. Brine shrimps

Briefly, the brine shrimps oocysts were incubated with artificial sea water that was prepared using sea salt (30 g/Lsea salt) under constant aeration for 48 h.

2.3. In vitro experiments

2.3.1. Brine shrimps bioassay

Preliminary investigation for biological activity of *S. longepedunculata* root extract was carried out using brine shrimps as earlier reported with slight modification[21]. Exactly, 1 mL containing 10, 48-hour-old nauplii were pipetted into each universal bottle. Increasing concentrations (1 mL) of 100, 200, 500, 1000 and 2000 μg/mL of the extract were added in three replications to give the respective effective concentrations. The controls were added dH_2O (0 μg/mL) and enumeration of organisms that were dead or alive was done using stereo-microscope (Bausch and Lomb, England).

2.3.2. Anthelmintic effects of S. longepedunculata root extract on L3 of H. contortus and H. polygyrus

Anthelmintic effect of the methanolic extract of *S. longepedunculata* on L3 larvae of *H. contortus* and *H. polygyrus* were done according to previous publication[22]. The larvae were exposed to three increasing concentrations (0.02-2.50 mg/mL) of the methanolic extract of *S. longepedunculata* in four replicates using 96 well micro-plates. The positive control wells were incubated with 1 mg/mL levamisole while the negative controls were incubated with dH_2O (0.00 mg/mL) at the same time period. Briefly, 50 μL of the L3 stage (7-33) of both *H. contortus* and *H. polygyrus* were introduced into the micro-plate. An equal volume (50 μL) of the extracts was added to each well to achieve the desired concentration of 0.02, 0.10, 0.50 and 2.50 mg/mL, respectively. The micro-plate wells containing the L3 stage of *H. contortus* and *H. polygyrus* were incubated for 24 h at room temperature.

2.4. Anthelmintic effects of S. longepedunculata root extract on adult worm of H. polygyrus in mice

Thirty albino mice (12.50-27.80 g) were used for the *in vivo* testing of the plant extract. The animals were acclimated for two weeks and segregated into five groups in clean Al cages. A negative control group (treated with dH_2O, 0.00 mg/kg bw), positive control group (treated with 100 mg/kg bw of pyrantel embonate) and 3 groups that were treated with increasing concentrations (500, 1000 and 2000 mg/kg bw) of the plant extract. During acclimation, water and food were provided *ad libitum* and the animals were screened of helminthe parasites and subsequently treated with 100 mg/kg bw of pyrantel embonate (strongid P, Pfizer limited, England) to preclude any round worm infection. Five days after the treatment, the mice were infected with an average of 250 L3 stage of *H. polygyrus* contained in 500 μL of distilled H_2O using esophageal tube connected to a variable pipette (Finnpipette, Digital Labsystems).

The mice were monitored daily till the 13 d post-infection after which fecal materials were collected for worm egg counts to guarantee the establishment of infection. Having established infection, the mice were then treated on the 18-21 d with 500, 1000 and 2000 mg/kg bw of the extract and the positive control group treated with 100 mg/kg bw of pyrantel embonate. The negative control group was treated with dH_2O (0.00 mg/kg bw).

2.5. Post-morten worm count

The post-morten worm count was done accordingly[19]. Following, treatments on the 18-21 d, the mice were killed by anesthetic (diethyl ether) overdose. Each mouse was anaesthetized with diethyl ether. The intestine was excised completely, slit open longitudinally by blunt dissection and placed in a nylon gauze (mesh size 1 mm) which was then immersed into a universal bottle containing pre-warmed Hank's balanced salt solution (pH 7.4). It was then allowed to stand at 37 ℃ for 1 h to allow outwards migration of the worms from the intestine through the gauze into the universal bottle. The worms were counted using stereo-microscope and hand lens including the worms lodged in the gauze.

2.6. Fecal egg count

The fecal egg count was calculated as egg per gram (epg) of the fecal material according to Mcmaster technique earlier described[15]. In brief, 1 g of thoroughly mixed feces was macerated and washed through a sieve (size 10 mm) with 15 mL of saturated salt solution (SG = 1.18, 400 g NaCl, 1000 mL dH_2O). The resultant suspension was mixed thoroughly with pasture pipette and equal volume of the suspension was introduced quickly under each of the two Mcmaster chambers (Hawksley, England) and viewed under a light microscope (10 × objective). The epg was calculated according to the equation: (number of eggs counted × total volume)/(volume counted × weight of fecal material).

2.7. Statistical analysis

The statistical analysis was done using Finney probit analysis (MS-DOS computer program). Comparisions were done by *t*-test and all data were reported as mean±SEM at

P<0.05 level of significance.

3. Results

3.1. Root extract

The freshly collected root of *S. longepedunculata* had a strong odor similar to menthol/methylated spirit. The dry pulverized coarse powder of the root of the plant causes persistent sneezing. The percentage yield of the methanolic extract upon evaporation to dryness was 7.13 % w/w dry matter and has a yellowish-brown color. When made into solution, the extract continuously froths suggesting the presence of saponin as one of its constituents.

3.2. Brine shrimps bioassay

The root extract of *S. longepedunculata* had a significant (P<0.05) biological activity on brine shrimps. Figure 1 shows a significant and dose-dependent increase in mortality on exposure to increased concentration of the extract resulting in 100% mortality at the highest concentration (1000 μg/mL). The LC_{50} was relatively high at 74.18 μg/mL.

3.3. Larvicidal effect of S. longepedunculata root extract

S. longepedunculata root extract showed significant (P<0.05), dose-dependent, larvicidal effect on *H. contortus* and *H. polygyrus* L3 stage compared to the controls. There was an initial increase in mortality from the lowest exposure concentration, followed by a plateau. Maximal effect of the extract on *H. contortus* and *H. polygyrus* was achieved at the highest concentration of the extract by 75% and 70%, respectively (Figure 2a and 2b).

3.4. Effect of S. longepedunculata on fecal egg count

The epg of infected mice was significant prior to treatment with the plant extract and the reference drug. The increase in epg was reduced only in the group treated with the reference anthelmintic. Pyrantel embonate significantly (P<0.05) reduced the epg to zero. However, the effect of the increased concentration of the extract on epg was similar to that of the untreated control group (Figure 3).

3.5. Post-mortem worm count

The maximal establishment of worm burden in the control was similar and comparable to all the groups treated with the extract. While treatment with extract of *S. longepedunculata* did not reduce the animal's twb significantly, treatment with pyrantel embonate resulted in complete eradication of worm burden. The twb in the animals treated to the highest concentration (2000 mg/kg bw) of the extract was not enumerated because of loss of the exposed animals towards the end of the experiment (Table 1).

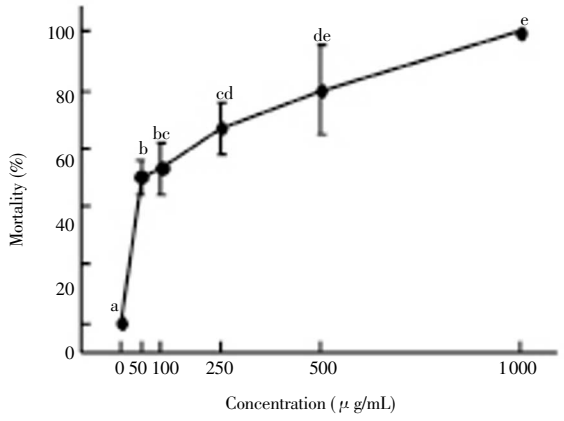

Figure 1. Lethal effect of the methanolic root extract of *S. longepedunuculata* on brine shrimps (*A. salina*) following treatment with equal volume of dH_2O (0 μg/mL, control) and *S. longepedunuculata* (50-1000 μg/mL) root extract.
Data are means±SEM. Points with dissimilar letters are significantly different from one another (P<0.05).

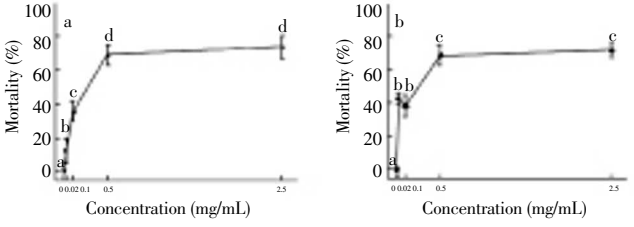

Figure 2. Larvicidal effect of the methanolic root extract of *S. longepedunuculata* on *H. contortus* (a) and *H. polygyrus* (b) L3 stages following treatment with equal volume of dH_2O (0 mg/ml, negative control), 1 mg/mL levamisole (positive control) and *S.*

Table 1
Post-mortem worm count after four consecutive days (18-21 d) of treatment of mice infected with *H. polygyrus* with dH_2O (0 mg/kg bw, negative control), pyrantel embonate (100 mg/kg bw, positive control) and 500-2000 mg/kg bw of root extract of *S. longepedunuculata*.

Treatment groups (mg/kg bw)		Mean female worm count	Mean male worm count	Total mean worm count	% worm establishment
dH_2O	0	83.00±0.60	114.70±7.90	197.70±7.90	79.20
S. longepedunuculata extracts					
	500	89.70±10.50	110.00±10.30	200.00±12.70	80.00
	1000	93.00±0.00	127.00±0.00	220.00±0.00	88.00
	2000	NE	NE	NE	NE
Pyrantel embonate		0.00±0.00	0.00±0.00	0.00±0.00	0.00

Data are means±SEM. Values without asterisks (*) are not significantly different from the control (P < 0.05). NE = not enumerated.

longepedunuculata (0.02-2.50 mg/mL) root extract (P<0.05).

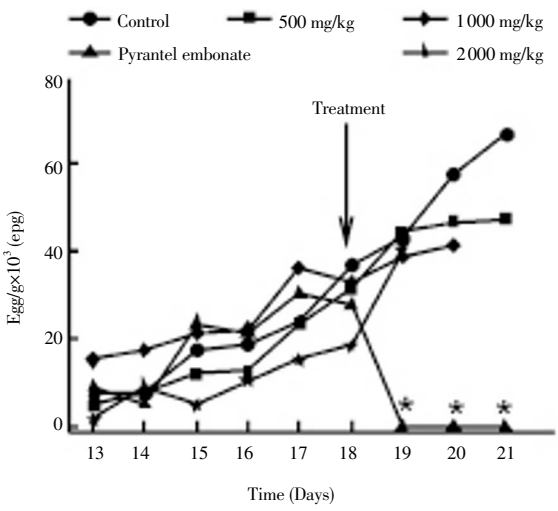

Figure 3. Fecal egg count of *H. polygyrus* before and after four consecutive days (18-21 d) of treatment of mice infected with *H. polygyrus* with dH_2O (0 mg/kg bw, negative control), pyrantel embonate (100 mg/kg bw, positive control) and *S. longepedunuculata* (500-2 000 mg/kg bw) root extract.

Values with asterisks (*) are significantly different from the negative control (P< 0.05).

4. Discussion

Preliminary study with Brine shrimps showed significant biological activity of the methanolic extract of *S. longepedunculata*. This bioassay was necessary to determine the potential of the extract for potent medicinal use prior to subjecting it to further biological analysis. The brine shrimps toxicity assay was also used in the determination of the toxicity of the plant extract. This simple, reliable, convenient and economical bioassay is often used in the assessment of medicinal plant for potential bioactive compounds and to substantiate their use in folkloric medicine[23,24]. The degree of mortality was proportionate to and correlated with the concentration of the exposure with the highest mortality observed in the highest exposure concentration. This suggests the presence of potent bioactive compounds in the crude extract. The LC_{50} on brine shrimps is considerably high indicating that the extract has a wide margin of safety compared to extracts from several medicinal plants[23]. Several studies have shown a wide range of biological activity from *S. longepedunculata* extracts including analgesia, antioxidant, anti-inflammatory and anti-depressant[13,14]. Suggestions abound that remedies made from this plant are bioactive against bacteria, fungi, viruses and protozoa re-emphasizing its popularity in tropical medicine practice[4,9,10].

The present study demonstrated that *S. longepedeunculata* root extract has larvicidal effect on *H. contortus* and *H. polygyrus* L3 stage of the parasites. Eradication of the intermediate stages of GIT nematodes is suggested to be very effective in the control of helminthes infection. This prevents the completion of the life cycle of the parasite and subsequent establishment of the adult worms in the respective predilection sites. This therefore prevents the propagation of the next generation of the parasites and perpetuation of the infection. Larvicidal effect of plant extract has previously been described in traditional medical practice. Similarly, dose-dependent, larvicidal effect of Pleiocarpa bicarpellata aqueous leave extract was observed on *Trichstrongylus columbriformis* and *H. polygyrus* L3 stage *in vitro*[22]. Unlike Njoku *et al*[22], the fecal egg count in the infected animals was significantly reduced by the extract while the post-morten worm count was similar in both the treated and non-treated groups indicating that the extract had ovicidal effect on the nematodes in mice. In another study, aqueous and methanolic root extract of *Adhatoda vesica* showed larvicidal, ovicidal as well as anthelmintic effect on the adult worms of *H. contortus* in sheep[25]. Further, treatment with crude aqueous and ethanolic extract of the aerial part of *Artemisia absinthium* resulted in significant reduction in epg and showed significant anthelmintic effect on adult *H. contortus* that was comparable to the reference drug, albendazole in sheep[26]. These suggest that there is huge potential for anthelmintic drugs from the plant community.

Gastro-intestinal nematodes infection are control or eradicated in humans and animals using a variety of anthelmintic agents of differing active principles and acting through different mechanisms of action[27]. Some anthelmintic drugs act against specific helminthe parasites or groups while others have a broader spectrum of activity acting against worms of different taxonomical groups and/or across various developmental life stages. Athelmintic effect studies on nematodes are better carried out in the natural habitat of the helminthes to eliminate confounding factors that may influence the outcome of the experiment. *H. polygyrus*, being a nematode of mice positioned this mice/GIT nematode infection model as a great model for studying effect of bioactive compounds on nematodes in mice[19,20,22].

Conclusively in the present study, *S. longipedunculata* demonstrated larvicidal effect on the L3 stage of *H. contortus* and *H. polygyrus* but not on the adult *H. polygyrus*. Additional work is suggested, using solvents of different polarities in the extraction process and treating a wide spectrum of helminthe organisms including the developmental stages from different taxo including trematodes, cestodes and nematodes for possible potential anthelmintic activity of *S. longipedunculata* extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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