

Anti-Helminthic Properties of Some Nigerian Medicinal Plants on Selected Intestinal Worms in Children (Age 5-13) in Ogurugu, South East Nigeria

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Abstract

Helminths parasites activity in children in Nigerian rural areas had caused a lot of deficiencies in the children from social to general well being of the body. The aim of this study is to investigate the anti-helminthic activity of *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana*, *Annona senegalensis*, *Ficus religiosa*, *Artemisia brevifolia*, *Calotropis procera*, *Pycnanthus angolensis*, *Nicotiana tabacum* and *Vernonia amygdalina* on these worms: *Ascaris lumbricoides*, *Strongyloides stercularis*, *Giardia intestinalis*, *Ancylostoma duodenale*, *Entamoeba histolytica*, *Enterobis vermicularis*, *Taenia saginata*, *Trichinella spp.*, *Necator americanus* and *Diphyllobothrium latum*. Aqueous (water) and ethanol extracts of leaves, stem bark and roots of the plants of concentration 20, 25, 50 and 100 mg/ml were used to test the worms for plant potency while piperazine citrate was used as control. Paralysis time and death were determined within 4 hours in the petri dish while unrestrained movements by the worms before and after extracts administration were recorded on a slow moving kymograph drum using the organ bath method. Time of paralysis and time of death were significantly reduced at all concentrations compared to the vehicle treated group ($P \leq 0.05$). The study showed that the extracts exhibited anti-helminthic activities on the intestinal worms, and can be used as an oral medication for these worms infestation in children.

Keywords: Aqueous extract; Anti-helminthic activity; Intestinal worm; Ogurugu community; Children

Introduction

Helminths are recognized as a major constraint to livestock production as well as blood loss in humans throughout the tropics [1,2]. They achieve these characteristics because majority of them live as pure obligate parasite in humans and other livestock of economic importance. These worms are grouped into two major phyla namely phylum Platyhelminthes (flatworms) and phylum Nematoda (roundworms) and they have developed various adaptive structures to survive in their hosts [3].

Plants extracts have been used since time immemorial for managing various diseases in traditional medicine especially in Africa and other developing worlds. Plants prescription has found that it's relevant as anti-cancer, anti-malarial, anti-coagulant, anti-histamine, antibiotic, food supplements, etc. However, the rapid spread of intestinal worm induced sickness in the rural populace in Nigeria, which had resulted into many deaths and physical impairments among children from age five to thirteen years, and negligence of orthodox medications by the these rural dwellers, justifies why this research was carried out.

The present research was therefore designed to scientifically validate some widely used ethno botanicals for their anti-helminthic activity in children of ages 5 to 13 years in Ogurugu Community, South Eastern Nigeria.

Materials and Methods

Collection of plant material

The plant material (Table 1) based on the information collected from ethno-medicinal survey was selected and procured from the local market/field and got authenticated from an expert in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The criterion for the selection of plants was seasonal availability of plants and previous work done on them i.e. if a plant is tested previously for anthelmintic activity.

Extract preparation

Plant material (in varying amount depending upon availability of plant) was dried under shade at a well ventilated place, cleaned of adulterants and ground to powdered form. The 10 plant materials were soaked in sufficient amount of 70% aqueous-methanol by cold maceration at room temperature for a total of 3 days. After that the filtrate was collected through a piece of porous cloth and filter paper and the plant materials re-soaked twice. The combined filtrate was concentrated in a rotary evaporator at 40°C under reduced pressure to yield thick and dark coloured crude extracts. These extracts were stored at -4°C until use and dissolved in distilled water on the day of the experiments to prepare stock solution and different dilutions for the purpose of evaluating pharmacological activity.

In vitro antihelminthic activity of extracts

Adult motility assay: Mature *Ascaris lumbricoides* from clinical isolates were used to determine the effect of Crude Aqueous Methanol Extracts (CAME) by method described previously by Iqbal et al. [4]. Briefly, the female mature worms were collected from freshly excreted faeces. The worms were washed and finally suspended in Phosphate Buffer Saline (PBS). A minimum of ten worms were exposed in three replicates to each of the following treatments in separate petri dishes at room temperature (25-30°C): 1) CAME at the rate of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 mg ml^{-1.2}) Abendazole 0.5 mg

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mL^{-1.3}) Phosphate Buffer Saline (PBS). The inhibition of motility and/or mortality of the worms kept in the above treatments were used as the criterion for anthelmintic activity. The motility was observed after 0, 2, 4, 6, 8 and 12 hour intervals. Finally, the treated worms were kept for 30 minutes in the lukewarm fresh PBS to observe the revival of motility. The number of dead and survived worms was recorded for each treatment.

Egg hatch test (EHT)

Egg recovery: Adult female *Ascaris lumbricoides* were collected after giving the longitudinal incision along the greater curvature of abomasums of naturally infected sheep [5]. The worms present in excreta or attached to the surface of guts were picked manually using forceps and placed in a bottle containing cool (4°C) PBS (pH 7.3) and later were triturated in pestle and mortar. The suspension was filtered through sieves of different sizes based on the nematode species into a bowl. Filtrate was centrifuged in Clayton Lane tubes for 2 minutes at 300x g and supernatant was discarded. Tubes were agitated to loosen the sediment and then saturated sodium chloride solution was added until a meniscus formed above the tube. A cover slip was then placed and sample re-centrifuged for 5 minutes at 130 rpm [6]. Cover slip was plucked off carefully from tubes and eggs were washed off into a conical glass centrifuge tube. Tube was filled with water and centrifuged for 2 minutes at 300 rpm. Supernatant was decanted and eggs were re-suspended in water. The eggs were then washed thrice in distilled water and adjusted to a 500 eggs mL⁻¹ using the McMaster technique [7].

Test procedure: Egg hatch test was conducted by the method described by Coles et al. [8]. Egg suspension of (0.2 ml; 100 eggs) was distributed in a 24 well multi-well plate (Flow Laboratories) and mixed with the same volume of different concentrations (0.25 to 8 mg mL⁻¹) of plant extracts (i.e., CAME) [8]. The positive control wells received different concentrations (0.09 to 3.0 µm mL⁻¹) of Abendazole (Systemax-ICI Pakistan, Ltd., 2.265%, w/v) in place of plant extracts while negative control wells contained the diluents and the egg solution. The eggs were incubated in this mixture at 27°C. After 48 hours, two drops of Lughole's iodine solution was added to stop the 37 eggs from hatching [9]. All the eggs (dead and embryonated) and hatched larvae in each well were counted. There were three replicates for each treatment and control.

In vivo antihelminthic activity of extracts

Faecal egg count reduction test (FECR): Study of animals: A total 120 local sheep of both sexes (≤1 year of age) weighing 18-25 kg having naturally acquired mixed parasitic infections of gastrointestinal nematodes were selected from Mr Ucheonwu's Farm Ogurugu. Infection was confirmed before the beginning of study by faecal examination of the animals, by the standard parasitological procedures [7]. The animals having higher than 500 eggs per gram of faeces were included in the experiment [10]. After selection of the animals, they were washed with an appropriate ecto-parasiticide. The animals were vaccinated against different bacterial/viral disease according to the routine. The sheep were kept on wood shaving and fed with fresh grass/fodder, concentrate (Anmol wanda[®]) and water ad libitum.

Treatment and follow-up procedures: Prior to the treatment, faecal samples were obtained by rectum from each animal, at least three times at an interval of three days. On each occasion, the number of eggs in the faeces according to the genus was determined by larval culture and identification was done by morphological characteristics described by MAFF [11] and Thienpont et al. [12]. The animals selected were

suffering from mixed gastrointestinal nematodes species including mainly *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Strongyloides stercularis*, *Necator americanus* and *Enterobis vermicularis*. On day zero, the sheep were allocated to eight groups of 4 animals each, according to the complete randomized design, taking into consideration their live weight [3]. These 38 groups were assigned different treatments as single dose for each plant as given below:

Group 1: Untreated control.

Group 2: Levamisole HCl (Nilverm[®] 1.5%, w/v; NIPRD Abuja, Animal Health Division) at 7.5 mg kg⁻¹ Body Weight (BW), served as treated control.

Group 3: Crude Powder (CP) at 1 g kg⁻¹BW.

Group 4: CP at 4 g kg⁻¹BW.

Group 5: CP at 8 g kg⁻¹BW.

Group 6: CAME at equivalent dose rate 1 g kg⁻¹BW of CP.

Group 7: CAME at the equivalent dose rate 4 g kg⁻¹BW of CP.

Group 8: CAME at the equivalent dose rate 8 g kg⁻¹BW of CP.

Measurements: Observation of clinical signs and/or death was undertaken daily. The body weight of the sheep was recorded weekly. Faecal egg counts per gram of faeces (EPG) were performed on each animal on days 0, 3, 6, 9, 12 and 15 post-treatment (PT) and were evaluated for the presence of worm eggs by salt floatation technique [11]. The eggs were counted by the McMaster method [7]. Egg Count percent Reduction (ECR) was calculated using the following formula:

$$\text{ECR (\%)} = \left\{ \frac{\text{pre-treatment EPG} - \text{post-treatment EPG}}{\text{pre-treatment EPG}} \right\} \times 100$$

Statistical analysis

The data from adult motility assay and *in vivo* experiments were statistically analysed using SAS software [13]. The results were expressed as mean ± standard error of mean (± SE).

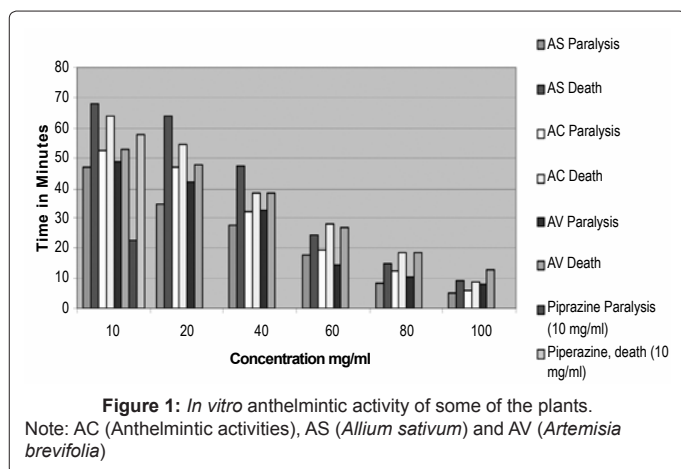
Results and Discussion

The plants demonstrated a very good potency on the all the selected worms (Table 1). Result shows that the higher concentration of the extracts produced paralytic effect much earlier and the time to death was shorter (Table 2). All the extracts and its fractions have shown significant antihelminthic activity among which aqueous extracts shows more prominent activity comparable to the standard anthelmintic drug, albendazole.

The plant inhibiting egg hatching (that is most potent) based on LC₅₀ was *Artemisia brevifolia* (2.13 µg mL⁻¹) followed in descending order of activity by *Calotropis procera* (2.41 µg mL⁻¹). The results suggest that all the ten plants have potential to inhibit egg hatching by the worms, indicating ovicidal activity by all the plants (Figure 1), and this was in support of the work by Sofowora [14], when he reported that ethno-medicinal prescription of plant for eradication of worms, had achieved great success with almost 63% of tropical plants showing anthelmintic activity.

Conclusion

The five plants *Annona senegalensis*, *Cucurbita mexicana*, *Calotropis procera*, *Allium sativum* and *Artemisia brevifolia* merit further consideration for the phytotherapy of helminthiasis as they showed



Plant species	Plant family	Part/s used
<i>Allium sativum</i> L.	Amaryllidaceae	Leaves
<i>Zingiber officinale</i> Ros.	Zingiberaceae	Rhizome
<i>Cucurbita mexicana</i> L.	Cucurbitaceae	Leaves
<i>Annona senegalensis</i> Pers.	Annonaceae	whole plant
<i>Ficus religiosa</i> L.	Moraceae	Leaves, roots
<i>Artemisia brevifolia</i> Wall.	Asteraceae	Whole plant
<i>Calotropis procera</i> At.	Asclepiadaceae	Leaves
<i>Pycnanthus angolensis</i> Wb.	Myristicaceae	Whole plant
<i>Nicotiana tabacum</i> L.	Solanaceae	Whole plant
<i>Vernonia amygdalina</i> De.	Asteraceae	Leaves

Table 1: Plants screened to evaluate anthelmintic activity. Note: Only few of the plants were represented in the results.

Test substance	Concentration (mg/ml)	Time Taken for Paralysis(P) and death (D) (In minutes)	
		P	D
<i>Ascaris lumbricoides</i>			
<i>Cucurbita mexicana</i>			
Aqueous extract	25	18.32 ± 0.58*	36.3 ± 0.89*
Aqueous extract	50	11.66 ± 0.63*	29.23 ± 0.58*
Aqueous extract	100	8.33 ± 0.47**	27.15 ± 1.73*
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-
<i>Annona senegalensis</i>			
Aqueous extract	25	-	-
Aqueous extract	50	-	-
Aqueous extract	100	-	-
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-
<i>Artemisia brevifolia</i>			
Aqueous extract	25	24.3 ± 0.33*	49.7 ± 2.02*
Aqueous extract	50	5.77 ± 0.58***	7.7 ± 0.32***
Aqueous extract	100	3.5 ± 0.41***	4.3 ± 0.72***
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-
<i>Allium sativum</i>			
Aqueous extract	25	18.32 ± 0.58*	36.3 ± 0.89*
Aqueous extract	50	11.66 ± 0.63*	29.23 ± 0.58*
Aqueous extract	100	8.33 ± 0.47**	27.15 ± 1.73*
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-
<i>Zingiber officinale</i>			
Aqueous extract	25	-	-
Aqueous extract	50	-	-
Aqueous extract	100	-	-
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-
<i>Calotropis procera</i>			
Aqueous extract	25	24.3 ± 0.33*	49.7 ± 2.02*
Aqueous extract	50	5.77 ± 0.58***	7.7 ± 0.32***
Aqueous extract	100	3.5 ± 0.41***	4.3 ± 0.72***
Albendazole	20	6.12 ± 0.55***	7.66 ± 0.33***
Vehicle	-	-	-

Only the most prevalent worms were represented, *Significant different; p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 students t-test.

Table 2: Anthelmintic activities of some selected plants on some intestinal worms.

good anthelmintic activities in dose dependent manner. Further bio-guided isolation of compounds from these five plants is, however, required to confirm which specific compounds are responsible for the observed anthelmintic activity. The observation that some of the extracts were inactive *in vitro* does not mean that they do not have activity on other cell types. Unless the concentration of pure active principle(s) in crude extracts is stated, care should be taken by the general population of Ogorugu (Nigeria) in oral application of crude extracts, because they are known to be converted to toxic metabolites by liver cells and subsequently cause liver cirrhosis.

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