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IN-VITRO ANTHELMINTIC ACTIVITY OF *ALCHORNEA LAXIFLORA*(BENTH.) EXTRACT AND FRACTIONS ON *TERRESTRIS LUMBRICOIDES*: NIGERIAN EARTHWORM

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ABSTRACT

Background: *Alchornea laxiflora* is a shrub which is widely distributed through Africa. It is one of the African medicinal plants used in treatment of some illments. **Objective:** We aim to investigate its antihelmintic activity of *Alchornea laxiflora* as part of effect to authenticate its oral scientifically. **Method:** Pulverized leaves (400 g) were cold macerated in claims methanol (1 liter) to obtain the crude extract. The extract was fractionated using liquid-liquid partitioning to obtain *n*-hexane, ethyl acetate and *n*-butanol fractions. The crude extract and its fractions were screened for phytochemical constituents using standard procedures. Acute toxicity (LD_{50}) of the crude extract was determined using Lorke's method. Adult earthworm (*Terrestris lumbricoides*) was used for the antihelmintics studies. The antihelminthic tests were carried out using standard procedure. Five petri dishes was labeled group 1 to 5 and five worms was introduced into each petri dish. Group 1 and 2 received 10 mls of 0.5 % CMC (negative control) and 20 mg/ml of Albendazole (positive control) respectively, group 3 to 5 received 12.5,25 and 50mg/ml of the crude extract. The time of paralysis and death of the worms were noted. Same was repeated for the fractions. **Results:** At all doses, the extract and fractions showed a significant ($p<0.01$) paralysis and the mean time of death was extremely significant ($P<0.01$). Time taken for paralysis and death was significantly greater ($p<0.05$) in the positive control group. **Conclusion:** Results obtained from this study proved that crude methanol extract and fractions of *Alchornea laxiflora* possess antihelminthic activity.

KEYWORDS: *Alchornea laxiflora*, antihelminthic, *Terrestris lumbricoides*, acute toxicity.

BACKGROUND

Helminths infections, generally known as helminthiasis is the infection of the human body with a parasitic worm such as roundworms and pinworms.[1] These parasitic worms can infect the tissues and intestines of a person. The worms usually only involve the intestinal tract but sometimes they may invade other organs. The type and severity of symptoms is determined by the type of worm and the part of the body infected.[2] Symptoms of helminth infections include nausea, vomiting, abdominal pains, confusion, and gastrointestinal bleeding. Helminthiasis causes a large threatening to the public health and lead to the prevalence of malnutrition, anemia, eosinophilia and pneumonia in developing countries.[3] Anthelmintics are drugs that expel parasitic worms (helminths) from the body, by either striking or killing the worms. Due to the high cost of currently available anthelmintic drugs and also the gastro-intestinal helminthes becomes resistant to the drugs; the treatment of helminthes diseases is one of the foremost problems.[4] Therefore the research on herbal remedies as alternative anthelmintics has been increased. As medicinal plants play a rich source of anthelmintic agents, they are used medicinally in different countries for the treatment of helminths infections.[5] Therefore, One practical way of developing cheaper and effective anthelmintics remains studying indigenous herbal remedies. There have been many reports, mainly from Africa, indicating the effectiveness of plant products against helminth infections in animals.[6] Thus, this study investigates the anthelmintic activity of *Alchornea laxiflora* on adult earthworm (*Pheretima posthuma*). *Alchornea laxiflora* is a plant used locally for the preservation of food items in Nigeria. It is a shrub which belongs to the family Euphorbiaceae which is widely distributed through Africa. *A. laxiflora* possesses numerous therapeutic benefits and has been used in the treatment of various disease conditions such as HIV/AIDS, malaria, diabetes, sickle-cell anemia, mental disorders and microbial infections.[6]

The leaf infusion of the plant is often used in folklore medicine as antimalarial.[7] The stem, especially the branches, is used in Nigeria as chewing sticks (local tooth brush) for cleaning teeth while the leaves are used to preserve kola nut and other perishable fruits and vegetables. Decoction of the leaves is usually administered to treat inflammatory and infectious diseases.[8] Oladunmoye and Kehinde[9] reported the use of *A. laxiflora* among the Yoruba tribe of Southwestern Nigeria for the treatment of poliomyelitis and measles. Other authors, Oloyede *et al*.[10], reported the antioxidant properties of the leaf extract of *A. laxiflora* in addition to its antimicrobial effects on four bacteria which are *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Farombi *et al*.[11] and fellow investigators also reported the antioxidant properties of *A. laxiflora* leaves and roots. The results obtained from their investigation indicate the presence of potent natural antioxidant which may be relevant in preservation of lipid food products. Antitoxicity, anticonvulsant, and sedative effects of the leaf extract of *A. laxiflora* in animal models have been reported by Esosa *et al*.[12] Despite its traditional claims, the efficacy of the leaves of *A. laxiflora* against helminths is not yet scientifically validated.

MATERIALS AND METHODS

Chemicals

Albendazole (Bendex) obtained from Cipla company, other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Experimental animals

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in vitro. Earthworms were collected from the damp soil at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka and washed with normal saline to remove all the faecal matter. *P. posthuma* with 6-8 cm in length and 0.3-0.5 cm in width was used for anthelmintic activity test. The worms were identified in the Department of Zoology, Faculty of Biological Sciences, Nnamdi Azikiwe University Awka. This organism was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.[8] Before initiation of experiment the earthworms were washed in normal saline.

Adult Swiss albino rats (190-230 g) was also used for the study and was obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University. The animals were fed with palletized feed (UAC feed, Nigeria) and had access to water *ad libitum*. Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. They were allowed free access to food and water. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub. No. 85 – 23 Revised 2011).

Collection and authentication of plant material

Plant collection and identification

Plant material: *A. laxiflora leaves* were collected from Agulu in Anaocha local Government area Anambra state Nigeria in November 2019. It was authenticated by a trained taxonomist, Mr Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimens (No. PCG 474/A/063) were deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka for future reference.

Methanol extraction

Five hundred grams (500 g) of the pulverized *A. laxiflora* root was macerated in one liter of methanol over a period of 48 hours. The mixture was sieved using porcelain cloth. It was further filtered with no. l Whattman filter paper. The filtrate was concentrated using rotary evaporator. It was further dried in a water bath at a temperature of 40° C to obtain the crude extract. The extract was then stored in a refrigerator for use.

Fractionation (Liquid-liquid fractionation)

The methanol extract (100 g) was subjected to liquid-liquid containing water (200 mL in 100 g of extract) were subjected to liquid-liquid partition successively with 1000 ml n-hexane, 1000 ml ethyl acetate and 500 ml n-butanol in increasing order of polarity. The fractions were filtered with Whatman no 1 filter paper and concentrated *in vacuo* using rotary evaporator at 40 °C to obtain the n-hexane fraction (HF), ethylacetate fraction (EF) and butanol fraction (BF). The extracts and all the fractions were stored in refrigerator between 0-4°C until they were used.

Phytochemical analysis

The phytochemical screening was carried out on the crude extract and fractions of *A. adiantifolia root* according to standard methods to identify the classes of bioactive compounds present.[13,14]

Acute-toxicity and lethality (LD_{50}) test

Acute toxicity analysis of the extracts was performed using Lorke's method.[15] Thirteen (13) Winster albino rats were utilized in this study. The test involved two stages. In stage one: the animals were grouped into three (3) different groups of three rats each. They were administered 10, 100, and 1000 mg/kg body weight respectively and in the second stage, 1600, 2900 and 5000 mg/kg body weight of the extract were administered. The administration of the extract was done orally. The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Anthelmintic activity on *Pheretima posthuma*

The anthelmintic activity was performed according to the method followed by Shelke *et al.*[16] All the experiments were carried out in Nigerian adult earthworms due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. They were collected from moist soil and washed with normal saline to remove all fecal matters. Five groups each containing six earthworms (*Pheretima posthuma*) of nearly equal size was taken for the experiment (n=5). Each type of dried extract was suspended in 1% w/v Carboxy Methyl Cellulose, prepared in normal saline water in three different concentrations (12.5, 25, 50 mg/ml). The test results were compared with standard drug Albendazole while normal saline water with 1% CMC was used as negative control. Worms were placed in petridish containing 15 ml of sample (drug) solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50 ◦C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour. Same was repeated for the fractions.

Statistical analysis

The results are presented as mean±SEM (Standard Error of Mean). Statistical analyses graphical representations of results for anthelmintic study were evaluated by one-way ANOVA following Dunnett's Multiple Comparisons test using Graph Pad Prism version 8.4 for windows. Graph Pad Software, San Diego California USA, the $p<0.005$ was considered to be statistically significant.

RESULTS

Result of phytochemical analysis of *A. laxiflora* leaves extract

The phytochemical analysis of ***A. laxiflora*** revealed that the crude extract is rich in alkaloids, flavonoids, tannins, terpenoids, proteins, carbohydrates and cardiac glycosides while saponins and steroids are absent.

Result of Acute toxicity test

In the acute toxicity and lethality test, results (Table 1) indicated no physical and behavioural change such as diarrhea, sleepiness, loss of appetite, coma or death. The LD_{50} was thus calculated to be ≥ 5000 mg/kg.

Table 1: Results of acute toxicity (LD_{50}) test.

Phase	Dose (mg/kg)	Mortality
Phase one	10	0/3
	100	0/3
	1000	0/3
Phase two	1600	0/1
	2900	0/1
	5000	0/1

Table 2: Effect of methanol extract on *Terrestris lumbricoides* (Earthworm).

Groups	Doses (mg/ml)	Initial weight of worm (mg)	Final weight of worm (mg)	Change in weight (mg)	Time of Paralysis (min)	Latent period before death (min)	Time of Death (min)
0.5% CMC	10	0.42±0.03	0.43±0.02	0.01	520.46±21.18	448.59	963.05±32.53
Albendazole	20	0.43±0.06	0.40±0.03	0.03*	123.42±13.43**	60.19**	183.61±16.58**
Crude extract	50	0.46±0.02	0.43±0.02	0.03*	15.33±3.44**	64.00**	79.33±7.32**
Crude extract	25	0.42±0.04	0.40±0.02	0.02^{ns}	16.33±3.47**	47.00**	63.33±4.26**
Crude extract	12.5	0.40±0.03	0.36±0.04	0.04**	14.16±0.59***	11.46***	25.62±3.67***

Results are expressed as mean ± SEM (n = 5). *p < 0.05, ** p < 0.01, *** p < 0.001 as compared with Control group (one way ANOVA followed by Dunnett t-test, 2 sided).

Table 2: Effect of fractions on *Terrestris lumbricoides* (Earthworm).

Groups and treatment (mg/ml)	Dose (mg/ml)	Initial weight of worm (mg)	Final weight of worm (mg)	Change in weight (mg)	Time of Paralysis (min)	Latent period before death (min)	Time of Death (min)
0.5% CMC	10	0.42±0.03	0.43±0.02	0.01	520.46±21.18	448.59	963.05±32.53
Albendazole	20	0.43±0.06	0.40±0.03	0.03*	123.42±13.43**	60.19**	183.61±16.58**
Ethylacetate fraction	25	0.44±0.02	0.40±0.05	0.04**	13.67±0.62***	9.00***	22.67±4.48***
Ethylacetate fraction	12.5	0.39±0.05	0.34±0.02	0.05**	13.14±0.31***	6.12***	19.26±3.39***
n-Hexane fraction	25	0.42±0.03	0.38±0.02	0.04**	13.67±2.15***	5.66***	19.33±3.87***

n-Hexane fraction	12.5	0.43±0.03	0.38±0.05	0.05**	10.0±2.11***	6.33***	16.33±3.26***
Butanol fraction	25	0.40±0.02	0.38±0.04	0.02	27.67±7.82**	40.33**	68.0±8.96**
Butanol fraction	12.5	0.44±0.05	0.42±0.03	0.02	38.67±6.69**	40.66**	79.33±6.33**

Results are expressed as mean ± SEM (n = 5). *p < 0.05, ** p < 0.01, *** p < 0.001 as compared with Control group (one way ANOVA followed by Dunnett t-test, 2 sided).

DISCUSSION

Phytochemical screening on the plant showed moderately presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, carbohydrates, protein and reducing sugars; this correlates with an earlier study done by Borokini *et al.*[17] These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. Some studies are available for anthelmintic activity of tannins, alkaloids, and flavonoids.[18,19] The presence of these phytochemicals may be responsible for the observed anthelmintic activity of plant extracts in the present study. Furthermore, tannins have been shown to interfere with coupled oxidative phosphorylation, thus blocking ATP synthesis in these parasites.[20] Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted (or adverse) effect is any effect that produces functional impairments in organs and/or biochemical lesions, which could alter the functioning of the organism in general or individual organs. Studies of acute toxicity however tends to establish the dose-dependent unwanted (or adverse) effect (s), which may take place and this, includes all information that is important in the assessment of acute toxicity including mortality.[21] The assessment of the lethal dose (LD_{50}) recorded no behavioral changes or mortality in the present study after administration of doses greater than 5000mg/kg. Hence, the leave sample has a high degree of safety.

A. laxiflora extracts and all the fractions produced a significant antihelmintics activity in a non dose dependent manne r as shown in Table 2 and 3. All the three concentrations of crude extract and fractions (50, 25 and 12.5 mg/ml) show better and potent antihelmintics activity than that of standard drug Albendazole. Albendazole is known to cause paralysis of worms so that they are expelled in faeces of man and animals. *A. laxiflora* leave extracts and fractions did not only demonstrated this property, but they also caused early death of worms at all concentrations compared to the standard drug. Thus findings from the current study revealed that the extracts and fractions have shown promising in vitro antihelmintics activity. This antihelmintics activity could be attributed to the bioactive compounds present in the plant acting jointly or separately. Our current study revealed broad antihelmintics activity of the extract and all three fractions and their broad activities are described for the first time. Tannin is one of the major active ingredients found in this plant material. Tannin is known to produce antihelmintics activity by binding to glycoprotein on the cuticle of the parasite which could be the possible mechanism of action in which *A. laxiflora* works. They hinder energy production in helminthes parasite by uncoupling oxidation phosphorylation causing death.[22] Such plant based treatments could be made part of an integrated management plan for control of helminthes in developing countries. Further studies are required to isolate and reveal the active compound contained in the crude extracts to establish the mechanism of action.

CONCLUSION

The present study assessed the methanol crude extracts and fractions of leaves of *A. laxiflora* possesses significant antihelmintics activity. Further studies are required for HPLC or LCMS analysis, to isolate and to characterize the bioactive constituents responsible of its antihelmintics activity**.**

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Conflict of interests

Declared none.

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