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Research Article

In Vitro Anthelmintic Potentials of *Bambusa vulgaris* (L.) Leaf Extracts Using Adult African Earthworm (*Eudrilus eugeniae*) from Southern Nigeria

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ABSTRACT

Bambusa vulgaris leafis used traditional among the aborigines of South-Western Nigeriato manage intestinal helminths with paucity of scientific evidence to the practice. This study was undertaken to evaluate the *in vitro* anthelmintic activities of the aqueous and methanolic extracts of *B. vulgaris*leaf (at doses 10, 20 and 30 mg/ml) using adult African earthworm (*Eudrilus eugeniae*) which has similar anatomy and physiology to human intestinal helminths. Albendazole (commercial anthelmintic drug) was used as a standard reference and saline as control. Both extracts displayed concentration-dependent and significant ($P<0.05$) vermicidal activities against the worms. The paralysis time of aqueous fraction of the plant at 10, 20 and 30 mg/ml concentrations were respectively observed at 14.42, 9.01 and 8.53 min followed by death at 21.03, 14.59 and 12.43 min post-exposure. Methanolic fraction caused paralysis at 12.33, 8.27, 7.52 min and post-exposure death time of 21.12, 14.57, 10.29 min. Both extracts (at 20 and 30 mg/ml) were more effective in promoting paralysis and death of the earthworms than the 10 and 20mg/ml treatments of the standard reference drug (Albendazole). The observations from this study therefore suggest that the bioactive principles of *B. vulgaris* leaf extracts possess significant anthelminthic activity against *E. eugeniae*, and possibly potent against human intestinal helminths that hassimilar anatomy and physiology to *E. eugeniae.* The ethno-medicinal claim of *B. vulgaris* as anthelminthic plant is thus substantiated.

INTRODUCTION

Helminthiasis or worm infections have continued to be the major health hazard to majority of people living in developing countries[1].Although these infections do not cause significant morbidity and mortality, when compared with many other parasitic infections, it has contributed to the prevalence of malnutrition, anaemia, diarrhoea, eosinophilia, pneumonia and poor health in children[1, 2]. Parasitic helminths also affect millions of livestock resulting in considerable economic losses in domestic and farmyard animals. The helminths which infect the intestine are cestodese.g. Tapeworm (*Taeniasolium*), nematodes e.g. hookworm (*Ancylostomaduodenale*), roundworm (*Ascarislumbricoides*) and trematodes or flukes (*Schistosomamansoni*and *S. haematobium*)[3].

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Over the years, helminthiasis had been control by the administration of synthetic anthelmintic drugs. Antihelmintics are drugs that expel parasitic worms (helminths) from the intestinal tract or tissues of the body by either stunning or killing them[4]. Despite the availability of these drugs, a large proportion of the world's population still does not have access to, or cannot afford to pay for them, particularly in remote rural areas in poor countries. Moreover, the continued usage of the currently available arsenal of anthelmintic drugs is also posing a major problem of drug resistance in several parasite species[5-7]. It is therefore pertinent to search for newer and inexpensive drugs that can serve as alternative or complementary agents to the available synthetic anthelmintic drugs. Several African plants have been screened and found to possess significant activity against helminth parasites[8-10]. With these promising results, there is still the need to search for more botanicals, which are readily available, accessible, multi-curative and relatively safe, so

as to complement the available arsenal of synthetic chemical and ultimately, increase the array of choices required for effective management of parasitic helminths.

Bambusa vulgaris L. (Family Bambusoideae) commonly known as "Bamboo", is a group of woody perennial evergreen plants ranging from 10cm - 40m in height and attaining maturity within five years, but flowering infrequently[11]. It is one of the most relatively fast growing species in tropical and subtropical areas, especially in the monsoon and wet tropics with about 91 genera and over 1000 species[12]. In Nigeria, bamboo is known with different tribal names, such as Oparun (Yoruba), Iko (Bini) and Atosi (Igbo), Ukute (Ukwuani), where it plays vital role in the traditional medicinal and socio-economics of the rural population[13, 14]. Ethno-botanically, the leaf and young shoot extracts of bamboo is used as emmenagogue, abortifacient, anthelminth, appetizer and for managing respiratory diseases, gonorrhoea, as well as skin rashes of HIV/AIDS[15]. The leaves of *B. vulgaris* are also applied in the treatment of fever, epilepsy, measles, alcohol poison, asthma, palsy, labour pains, hypertension, vulnerary and febrifuge to heal the wounds and control diarrhoea in cattle[16-20]. The effective ingredients of bamboo leaf was reported to include includes flavones, phenolic acid, lactones, amino acids and micronutrient[21]. Phytochemical evaluation of its methanolic leaf extract revealed the presence of alkaloids, tannins, phenolics, cardiac glycosides, saponins, flavonoids, reducing sugars and anthraquinone[14]. Recently, the leaf extract was reported to possess abortifacient, antiviral, antimicrobial and antiplasmodial properties[14, 16, 22, 23].

Despite these arrays of documented reports of *B. vulgaris,* currently available literature revealed that there is paucity of information on the scientific proof to buttress the acclaimed potentials of this plant as traditional remedy for intestinal helminths. The present study was therefore undertaken to investigate the in vitro anthelminthic activities of the aqueous and methanolicextract of *B. vulgaris* leaf using adult African earthworm (*Eudriluseugeniae*) which has similar anatomy and physiology to human intestinal helminths. This will thus establish the pharmacological basis for its folkloric use as treatment for intestinal worm in southern Nigeria.

MATERIALS AND METHODS

Drugs and Chemicals

Albendazole (Micro Lab. Ltd., Goa) and methanol (BDA chemical Ltd, Poole England), Normal saline was used during the experimental protocol.

Experimental animals

Adult African earthworms (*Eudriluseugeniae*) of 5-8 cm in length, 0.1-0.3cm in width and weighing 0.8 - 4.0 g were used for all experimental protocol due to their general anatomical and physiological resemblance with the intestinal roundworms parasites of human beings[24]. All the earthworms were sourced from moist soil within Novena University campus and washed with normal saline to remove all faecal and waste matters. They were authenticated at the Department of Biological Sciences, Novena University, Ogume. Ethical approval was obtained from the Animal Ethics Committee of the Department of Health Sciences, Delta State University Abraka, Nigeria and experimental procedures were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental animals[25].

Collection and Preparation of Plant material

Fresh leaves of *Bambusa vulgaris* were collected at Amai town in Ukwuani Local Government Area of Delta State, and were identified at the Moist Forestry Research Station, Forestry Research Institute of Nigeria, Benin, Edo State, Nigeria, where voucher specimens were deposited for future reference. The collected leaves were washed properly with tap water and rinsed with sterile distilled water before they were air-dried at ambient temperature (28±2°C) to constant weight for about 14 days. The dried materials were reduced to coarse form using a pestle and mortar and further reduced to very fine particles with an electric blender (Super Search Model 2815). The powdered sample obtained was labeled and stored in a polyethylene bag until needed for analysis.

Extraction of plant material

Four hundred grams (200g) of the powdered plant material was successively extracted by maceration in 500ml each of sterile distilled water and methanol for 72hours with intermittent manual shaken. Each extract was filtered through cheese cloth and Whatman No 1 filter paper and then subjected to rotary evaporation at 40^{o}C to obtain dark greenish

sticky mass extract with yields of 10.24 and 8.97%w/w respectively.

Anthelmintic Activity

The method of with slight modification was adopted for the in vitro anthelmintic assay of the plant extracts[26]. The earthworms were divided into eight different groups, each group containing six worms and placed in different Petri Dishes (9cm diameter).The first six groups of worm was treated with 50 ml formulations of each extract solutions (at concentrations of 10-30mg/ml in normal saline), while the last two groups were treated with 50ml each the standard drug (at concentrations of 10-30 mg/ml, Albendazole) and normal saline (control). Observations were made for the time taken for paralysis (Paralysis was said to occur when the worms did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water at 50^0C , followed with their body colours fading away).

Statistical analysis

The results on gastrointestinal transit time were expressed as mean ± S.E.M. Differences between means were analysed using one-way analysis of variance (ANOVA).Values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Helminths or worm infestations continue to be one of the most prevalent diseases and serious public health problems worldwide. The major control strategy adopted against helminths or worm parasiteshas been the use of anthelminthics. Anthelmintics are the drugs used toeradicate or reduce the number of helmintic or worm parasites in the intestinal tract ortissues of human and other animals. However, the current development of resistance to most commercially available anthelmintic clearly suggest that control programs based exclusively on their use are not sustainable.

In this study, the aqueous and methanolic extracts of *B vulgaris* leaf were found to exhibit dose-dependent and significant ($P<0.05$) in vitro anthelmintic activity against earthworms with paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. From Table 1, the paralysis time of the aqueous fraction of the plant at 10, 20 and 30 mg/ml concentrations were respectively observed at 14.42, 9.01 and 8.53 min and death at 21.03, 14.59 and 12.43 min post-exposure. The methanolic fraction on the other hand caused paralysis at 12.33, 8.27, 7.52 min and post-exposure death time of 21.12, 14.57, 10.29 min (Table 1). When compared with the standard reference drug (Albendazole) at 10 and 20 mg/ml, both extracts (20 and 30 mg/ml) were more effective in causing promoting paralysis and death of the earthworms as well. However, the promotion of paralysisand loss of motility by albendazole at 30 mg/ml was similarto those of bothextracts.

The predominant effect of albendazole on the wormis to cause a flaccid paralysis that result inexpulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation andflaccid paralysis. Also albendazole has been reported to cause paralysis in worm by disrupting the microfilaments, microtubules and β-tubulins component of their cytoskeletal structure[27]. The extracts of this plant might have exerted their anthelmintic effects on the test worm in a similar manner to albendazole. The mechanism of the anthelmintic activity of *B. vulgaris* leaf extracts could possibly be due to the disruption of the permeability of the cell membrane of earthworm causing vacuolisation and disintegration of the teguments. Another possible suggestion for the observed paralysis and death of the worms is the binding of the bioactive principles of the extract to glycoproteins on the cuticle of the worms causing disruption of cell membrane integrity. Earlier researchers reported that anthelmintic agents act by binding to the free proteins in the gastrointestinal tract of the host animal or glycoproteins on the cuticle thereby causing disruption of cell membrane integrity, disruption of the metabolic pathways of the worms[28-30].

Several secondary metabolites of plant extracts have been noted to play key roles inanthelmintic activities. Our previous study on the preliminary phytochemical analysis on the methanolic extract of *B. vulgaris* leaf revealed the presence of flavonoids, saponins, cardiac glycosides, alkaloids, anthraquinones, phenolic compounds, reducing sugars and tannins[14]. Earlier researchers have implicated some of these phytochemical in the anthelminthic activities of medicinal plants. Tannins, a polyphenolic compound, have been reported to produce anthelmintic activity[31].

Table 1: Anthelmintic activity of various extracts of B. vulgaris leaf and Albendazole against Eudriluseugeniae (African earthworm)

Treatment	Concentration (mg/ml)	Paralysis Time (minute)	Death Time (minute)
Normal saline (control)	10ml	--	--
Albendazole (Reference drug)	10	18.01 ± 0.17	26.27 ± 0.05
	20	10.24 ± 0.05	16.22 ± 0.10
	30	7.03 ± 0.25	8.05 ± 0.15
Aqueous Extract	10	14.42± 0.05*	21.03± 1.11*
	20	9.01 ± 0.11*	14.59± 0.06*
	30	8.53± 0.25*	12.43 ± 0.05*
Methanol Extract	10	12.33 ± 0.58*	21.12 ± 0.45*
	20	8.27± 0.45*	14.57± 0.10*
	30	7.52± 0.57*	10.29± 0.25*

Each value represents Mean ± SEM (N=6). *Mean value significantly different (P < 0.05) compared with control group.

Some synthetic phenolic anthelmintics like niclosamide, oxyclozanide andbithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidativephosphorylation. Alkaloids were reported to cause paralysis by acting on the central nervous system and permeability of the cell membrane of worms[32, 33]. Saponins are known toinduce and disrupt the selective permeability of the cell membrane in a similar way to the anthelmintic drug. Therefore, it is possible that the relative potent anti-worm activity of *B. vulgaris* leaf in this study could be attributed to the interaction of one or more of the bioactive components in the extracts.

CONCLUSION

This study has demonstrated that the aqueous and methanol leaf extracts of *B. vulgaris* possess significant in vitro ant-worm activity at the tested concentrations. Thus, the wormicidal activities of the extracts against earthworms suggest that it could be effective against parasitic helminths of humans and animals, since they have similar anatomy and physiology. The experimental evidence obtained in this laboratory model could provide a rationale for the traditional use of this plant as anthelminth. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of actions. Also, studies on the toxicity, evaluation of the effect *in-vivo* condition and the establishment of adequate doses for humans and animals are recommended.

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