

IN-VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF CRUDE EXTRACT OF THE LEAVES OF DALBERGIELLA WELWITSCHII

T.SHADE OLUSEGUN-JOSEPH, L.NWANNEKA OFODILE, TOLULOPE OGUNTOKE

Department of Biological Sciences, School of Science, Yaba College of Technology, Yaba, Lagos, Nigeria. Email: shadefame@gmail.com

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ABSTRACT

Dalbergiella welwitschii commonly known as West African blackwood belongs to the family fabaceae. In the present research study, crude methanolic extract from leaves of *Dalbergiella welwitschii* was investigated for its anthelmintic activity against earthworms (*Pheretima posthuma*) and liver flukes (*Fasciola species*). Three concentrations (100mg/ml, 200mg/ml and 300mg/ml) of extract were tested *in-vitro* for anthelmintic potency by determination of time of paralysis and time of death of worms. Albendazole (10mg/ml) was used as standard reference. Results showed that the extract 100mg/ml – 300mg/ml caused paralysis and death of liver flukes between 23:03 to 36:25 minutes and 38:15 to 81:06 minutes respectively. While the standard drug albendazole paralyzed and caused the death of liver flukes after 44:06 and 95:03 minutes respectively. Also, the extract of *D. welwitschii* 100mg/ml – 300mg/ml showed anthelmintic activity against earthworms by paralyzing and causing it death between 37:46 to 52:20 minutes and 101:8 to 180:10 minutes respectively. While the standard drug albendazole paralyzed and caused the death of earthworms after 150:21 and 280:45 minutes respectively. There was clear indication that anthelmintic activity of methanolic extract of *D. welwitschii* was more potent against *Pheretima posthuma* than on *Fasciola species* and comparable to the standard. This study confirms the traditional claim of *Dalbergiella welwitschii* as an anthelmintic, as the crude methanolic extract displayed anthelmintic activity against the worms used in this study.

Keywords: *Dalbergiella welwitschii*, Paralysis, Death, *Pheretima posthuma*, *Fasciola species*

INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Majority of infections due to helminthes are generally restricted to tropical regions causing enormous hazard to health and contributing to prevalence of under-nourishment, anemia, eosinophilia and pneumonia[1]. Helminthes also affect domestic animals and livestock, causing considerable economic loss[2]. Parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms which results in weakness, loss of appetite, decreased productivity[3]. The major control strategy adopted against helminthes parasite is the use of anthelmintics[4]. The gastrointestinal helminthes are becoming resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases[5]. Indiscriminate use of synthetic anthelmintics can lead to resistance by parasites[6]. Traditional system of medicine reports efficacy of several natural products eliminating helminthes[7]. In view of this, several researchers have undertaken studies to evaluate folklore medicinal plants for their proclaimed anthelmintic efficacy[8]. Considering this *Dalbergiella welwitschii* has been evaluated for its anthelmintic activities.

Dalbergiella welwitschii in the bean family (fabaceae) which is commonly known as West Africa black wood is a leguminous flora of West Africa. It has geographical records in Angola, Cameroon, Central African Republic, Gabon, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo, Taire. It has a wide range of uses such as abortifacients, ecbotics, arthritis, rheumatics, stomach trouble etc. The bark is used in treatments of cutaneous and subcutaneous parasitic infections. The root and leaf is used as vermifuges. Resistance of parasites to existing drugs and their high cost warrants the search for newer anthelmintic molecules [8], thus the reason for this work.

This paper therefore, reports the effect of the leaves of *Dalbergiella welwitschii* on *Pheretima posthuma* and *Fasciola species* in order to evaluate its claimed anthelmintic activity. The phytochemical screening of the plant was also done to determine its chemical constituents.

MATERIALS AND METHOD

Plant material: The fresh leaves of *Dalbergiella welwitschii* was collected from a forest in Osun State, Nigeria. The leaves were authenticated by

Herbarium of Botany Department, University of Lagos, Nigeria. A voucher specimen (No: LUH3913) was deposited in the center.

Preparation of Plant Crude Extract

The leaves were oven dried at 20°C and grinded to coarse powder. This was stored in an air tight container and used for further successive extraction. Dried and powdered leaves of *Dalbergiella welwitschii* (300g) was extracted with 900ml of methanol by soaking overnight under the fume cupboard and filtered into a weighed beaker and the residue discarded. The extract was constituted under the fume cupboard to obtain the methanol extract. The extract was reconstituted into 100mg/ml, 200mg/ml and 300mg/ml in 90% methanol. The methanol extract (0.5g) was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by standard methods [9]. The screening covered mainly alkaloid, tannins, saponins, phlobatannins, glycosides, anthraquinones and steroid.

Anthelmintic Activity

Adult earth worms (*Pheretima posthuma*) and liver flukes (*Fasciola species*) were used to evaluate anthelmintic activity in vitro. Earth worms were collected from moist soil and washed with distilled water to remove all debris and the liver flukes were obtained from liver of freshly slaughtered animals at the local slaughter house and kept in phosphate buffer saline solution (PBS). The earthworm and liver flukes were identified in the Department of Zoology, University of Lagos and service of the veterinary practitioners was utilized to confirm the identity of the worms.

The anthelmintic assay was carried out as described by Ajaiyeoba et al, 2001 [10] with minor modifications. The assay was performed in vitro using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal round worm parasites (*Ascaris lumbricoides*) of human being[11,15]. Because of easy availability; earthworms have been widely used for initial evaluation of anthelmintic compounds in vitro [12,13]. Use of liver flukes (*Fasciola species*) as a suitable model for screening of anthelmintic drug have been advocated earlier[14]. Test samples of the crude extract were prepared at concentrations of 100, 200 and 300 mg/ml in distilled water for earthworms and PBS for liver flukes.

Six worms (same species) of approximately equal size were placed in different petri-dishes containing 20ml of different concentrations of extract (100, 200, 300mg/ml). Albendazole (10mg/ml) was used as reference standard. Phosphate buffer saline solution and distilled water were used as control for liver fluke and earthworm respectively.

All test solutions and standard drug solutions were prepared freshly before starting experiments. Observations were made for time taken for paralysis; this was noted as when no movement of any sort could be observed except when shaken vigorously. Time taken for deaths of worms was also recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C).[11,13]

RESULT AND DISCUSSION

Preliminary phytochemical screening of crude extract of the leaves of *Dalbergiella welwitschii* revealed the presence of Alkaloids, saponins, tannins, steroid and glycosides. The presence of phyto-constituents is reported in Table 1.

Table 1: Preliminary Phytochemical Screening of Methanol Extract of *Dalbergiella Welwitschii*

Phytoconstituents	Crude Extract Inference
Alkaloids	+
Saponins	+
Tannins	+
Phlobatannins	-
Anthraquinines	-
Glycosides	+
Steroid	+

Key: + = presence; - = absence.

Table 2: In-vitro anthelmintic activity of methanolic extract of *Dalbergiella welwitschii*

Extract	Conc. (mg/ml)	<i>Pheretima posthuma</i>		<i>Fasciola species</i>	
		TP (Min.)	TD (Min.)	TP (Min.)	TD (Min.)
Methanol	100	52.20 ± 0.2	180.10 ± 0.01	36.25 ± 0.4	81.06 ± 2.78
		44.52 ± 0.2	153.06 ± 0.02	35.84 ± 0.75	60.27 ± 0.37
Methanol	200	37.46 ± 0.01	101.8 ± 0.08	23.03 ± 0.33	38.15 ± 0.15
		150.21 ± 2.11	280.45 ± 0.17	44.06 ± 0.02	95.03 ± 0.01
Albendazole	10	-	-	-	-
Control	-	-	-	-	-

Key: TP = paralysis; TD = death, each value represents mean ± SEM (N = 6)

The crude extracts of the leaves of *Dalbergiella welwitschii* produced a significant anthelmintic activity on *Fasciola* in a dose dependent manner as shown in Table 2. The methanol extract of the leaves of *Dalbergiella welwitschii* demonstrated paralysis as well as death of liver flukes (*Fasciola*) in a less time as compared to albendazole especially at higher concentration 300mg/ml. The potency of the extract against the worms also increased with higher concentration and time.

The phytochemical screening of the methanolic crude extract has revealed the presence of tannins. Tannins have been shown to produce anthelmintic activity [16]. Chemically tannins are polyphenolic compounds [17]. Some synthetic phenolic anthelmintics like Niclosamide, Bithionol, and Oxytoclozamide have been shown to interfere with energy generation in helminth parasites uncoupling oxidative phosphorylation [18]. It is possible that tannins contained in the extract of *Dalbergiella welwitschii* produce similar effects on liver flukes. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro-intestinal tract of host animal or glycoprotein on the cuticle of parasite and cause death. [19,20]. Other plant metabolites such as alkaloids, saponins and glycosides found in the extract could also be responsible for the anthelmintic effect of the extract.

CONCLUSION

Results reported in the present work constitute rational evidence and a scientific basis to justify and support the folklore claims of the potential anthelmintic activity of leaves of *Dalbergiella welwitschii*. Further research should be carried out to isolate and characterize the active constituents responsible for such activity, using *in vivo* models and establishment of the possible mechanisms of action.

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