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Anthelmintic thin layer chromatographic fractions of the leaves of the herbal plant *Dalbergiella welwitschii*

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Abstract

Background: *Dalbergiella welwitschii* known as ewosho-elomoso in Yoruba language is a herbal plant used in the treatment of different types of worm diseases in man and cattles but enough scientific proof is lacking in this regard. Helminths diseases of human and animals are among the toughest infections to control in our world today.
Objective: The thin layer chromatographic fractions of the water and methanol extracts of *Dalbergiella welwitschii* were evaluated for their anthelmintic activities against earthworms (*Pheretima postuma*) and liver flukes (*Fasciola hepatica).*
Results: Results showed that the water extract eluted with methanol at Rf 0.86, methanol extract eluted with methanol at Rf 0.00 and methanol extract eluted with Butanol: Ethanol: Water at Rf 0.00 caused paralysis and death of liver flukes between 10:79 and 19:47 minutes, 12:86 and 21:29 minutes and 13:26 and 28:86 minutes respectively while the standard drug albendazole paralyzed and caused death of liver flukes in 46:66 and 129:31 minutes. The methanol extract eluted with Butanol: ethanol: Water at Rf 0.27 and water extract eluted with Butanol: ethanol: Water at Rf 0.37 caused paralysis and death of earthworms between 03:76 and 04:56 min and 05:47 and 06:21 min. The standard drug albendazole caused paralysis and death in 482:10 and 720:35 min. Lack of significant alterations in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphate, Creatinine and glucose suggests that subchronic administration of extract neither altered hepatocytes and kidneys of rats nor the normal metabolism of the animals. There was also no significant increase in the level of heamatological parameters in all treated groups compared to the control. Some of the thin layer chromatographic fractions of the herbal plant were highly significantly more anthelminthic than the standard drug.
Conclusion: It is concluded that the water and methanol extracts of the leaf of *Dalbergella welwitschii* are anthelmintic, safe for consumption and could be used in the formulation of novel anthelminthic drug.

Keywords: Anthelmintic, *Dalbergiella*, Thin layer chromatography, worms, Nigeria

INTRODUCTION

Infections caused by helminths are among the most stubborn infection and a foremost progressive disease plaguing humans and animals around the world. They pose a great threat to public health and add to the prevalence of pneumonia, malnutrition and anaemia in developing countries (Bundy, 1994; Ajaiyeoba et al., 2001). The helminths as parasites live in human body in intestinal tract and can also found in tissues, as their larvae migrate towards to the tissues (Tripathi, 2003). Most disease caused by helminthes are of long-lasting, devastating nature; they probably cause more injury and greater economic and social deficit among humans and animals than parasites.

Dalbergiella welwitschii (Baker F.) commonly known as the west African black wood is a shrub or climber to 17m of dry deciduous forest, riverine forest fringing forest and abandoned farmland. It is used as medicine for arthritis, stomach troubles, ecbolics, rheumatism, and arbortifacients. The root-bark and leaf are used as antidotes. The product from its bark is used for resins and exudations-gums the leafy stem is used as medicine against cutaneous and subcutaneous parasitic infections. The root and leaf are used as vermifuges. The wood is used for household, pastimes-carving, games domestic and personal items, musical instruments, and toys (Shalaby 2013, Olusegun-Joseph et al., 2013).

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Animal test results often represent the only means by which toxicity in humans can be effectively predicted. Animal tests for toxicity are conducted before human clinical investigations as part of the non-clinical investigations laboratory tests for pharmaceuticals but human testing is rarely conducted for pesticides and industrial chemicals (Ghule et al., 2011).

The important worm control strategy throughout the world is chemical control coupled with improved management. However, development of resistance in Helminthes against conventional anthelminth is a foremost problem in treatment of helminthes diseases (Shalaby 2013). It is therefore, important to search for alternative remedies against gastrointestinal nematodes, hence this work on anthelminthic activity this plant.

This paper therefore reports the effect of the thin layer chromatographic fractions of the aqueous and methanol extracts of the leaves of the herbal plant *Dalbergiella welwitschii* on two worms Adult earthworms (*Pheretima posthuma*) and liver flukes (*Fasciola hepatica*)

MATERIALS AND METHODS

The fresh samples of leaves of *Dalbergiella welwitschii* were collected from Ikire in Osun State, Nigeria. Ground plant material (200g) was soaked in 2 liters of distilled water overnight to prepare crude aqueous extract while the methanol extract was prepared by soaking with 2 litres of methanol. The solution was then filtered using a muslin cloth and filtered with Whatman filter paper. The filtrates were concentrated by freeze-drying and the extracts were stored at 4^0C until use.

Adult earthworms *(Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2cm in width were used for all the experimental protocol, due to its anatomical and physiological resemblance with intestinal roundworm parasite of human beings (Raghavamma and Rama Rao, 2010; Olusegun-Joseph *et al.*, 2013). Liver flukes (*Fasciola hepatica)* were obtained from freshly slaughtered cows at Bariga abattoir in Lagos State, Nigeria. It was kept in phosphate buffer saline solution (PBS).

The liver flukes were authenticated in the laboratory by the parasitologist Mrs Olusegun-Joseph, department of biological science, Yaba College of Technology.

Thin layer chromatographic techniques

A vertical line was ruled, 1cm from the base of each plate using pencil. Short horizontal marks (0.5) were then made below each line. An aliquot of 10µl of each sample was applied on the marks along the line on the pre-coated TLC plates (1.05554 aluminum sheets, 20 by 20cm, silica gel 60F254,Merck).TLC plates were developed in TLC tanks using the following mobile phases: (i)Methanol extract with methanol (ii)Methanol extract with Butanol: ethanol: water (4:5:1) (iii) Water extract with Methanol (iv) Water extract with Butanol: ethanol: water(4:5:1). Plates were removed from tank after solvents have moved up the plates. Solvent fronts were marked leaving about 1cm beyond the front. Plates were left in the fume cupboard to dry. After developing the plates, they were viewed under the UV lamp (254nm). The process was repeated to get enough extract and the different bands were noted and corresponding bonds were marked (Ofodile et al. 2005)

The Rf of each band was calculated and recorded. The various bands observed were then scrapped with a blade into a sterile beaker; bands with the same Rf were scrapped into the same beaker and labeled. Methanol (40ml) was added into each beaker to dissolve the fractions; these were then filtered into sterile weighed labeled vials. The filtrates were concentrated by evaporation in a water bath. The extract was reconstituted using distilled water and used for the bioassay (Ofodile et al. 2005)

Bioassay

The anthelmintic activity was carried out as described by Ajaiyeoba et al. (2001) with minor modifications.

Animal Toxicity Test

Animal collection

Swiss albino mice (20-25g) of either sexes were obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Idi-Araba and were taken to the Animal Facility, Yaba College of Technology. They were maintained on standard animal diet (Feeds Nigeria Limited) purchased from Mushin market, Lagos, Nigeria and provided with water ad libitum. They were allowed to acclimatize for seven days under standard laboratory conditions before the experiment. Each rat in a study groups were individually housed in plastic cages at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h light –Dark cycle.

Acute and subchronic toxicity test

Acute and Subchronic toxicity tests were carried out using Swiss mice and Wister rats respectively according to the method of Ogbonnia et al. (2011) The Swiss mice (12-19g) and Wister rats (120-150g) of either sex were obtained from the laboratory animal center, College of Medicine, University of Lagos, idi-Araba. The toxicity was carried out using thirty five male and female Swiss albino mice. They were randomly distributed into one control group and six treated groups, containing five animals per group. After fasting the animals over-night, the control group received 0.4 mL of water orally and each treated group received the extract doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg/g body weight (bwt) orally. The animals were observed at 0hr, 1hr, 2hrs, 4hrs, 6hrs, 8hrs, 24hrs and 72hrs after administering for any behavioral or clinical signs of toxicity.

Standardized diagnostic kits (Lankit) were used for spectrophotometric determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate amino-transferase (AST), creatinine, alkaline phosphatase total proteins and urea. The haematological parameters determined were packed cell volume (PCV), Mean Corpuscular Heamoglobin

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Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin (MCH), White Blood Cell (WBC), Platelet count, Haemoglobin (Hb)

Statistical analysis

Data on the different parameters were analysed by statistical analysis using SPSS 12.0. Statistics software. The results are expressed as mean± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) with turkey test to evaluate significant differences between groups. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The results of the anthelminthic activities of the thin layer chromatographic fractions of the different solvent extracts of the herbal plant are shown in tables 1 and 2.

Table 1: In vitro anthelminthic activity of TLC fraction of the Methanolic extract of *Dalbergiella welwitschii* against worms

Fractions and Solvent elution	Earthworm (*Pheretima postuma*)		Liver fluke (*Fasciola hepatica)*	
	Time of Paralysis (min)	Time of Death (min)	Time of paralysis (min)	Time of Death (min)
Methanol				
$Rf_1 = 0.00$	682:02	886.92	12.86	21.29
$Rf_2 = 0.50$	24:31	61.:40	24.29	57.73
$Rf_3 = 0.87$	81:40	108.17	22.44	67.35
But:Eth:Water				
$Rf_1 = 0.00$	After 24 hrs	After 24 hrs	13.26	26.86
$Rf_2 = 0.27$	03:76	04:56	13.45	38.51
$Rf_3 = 0.77$	After 24 hrs	After 24 hrs	12.79	53.78
$Rf_4 = 0.79$	After 24 hrs	After 24 hrs	15.04	98.18
$Rf_5 = 1.00$	After 24 hrs	After 24 hrs	13:58	75:20
Albendazole	482:10	720:35	22.12	60.23

Table 2: In vitro Anthelmintic activity of TLC fractions of water extract of *Dalbergiella welwitchii* against worms

Solvent elution Fractions	Earthworm (*Pheretima postuma*)		Live fluke *(Fasciola hepatica*)	
Methanol	Time of Paralysis (min)	Time of death (min)	Time of paralysis (min)	Time of Death (min)
Rf1 = 0.00	After 20hr	The next day	90:18	129:16
Rf2 = 0.61	1200:34	After 24hr	22:26	147:68
Rf3 = 0.86	After 20 hr	After 24hr	10:79	19:47
But:Eth:water				
Rf1 =0.00	After 24hr	After 24hr	18:71	105:06
$Rf_2 = 0.37$	05:47	06:21	38:32	184:41
$Rf_3 = 0.79$	After 24hr	After 24hr	26:37	57:54
$Rf_4 = 1.00$	After 24hr	After 24hr	31:85	42:24
Albendazole	482:10	720:35	46:66	129:31

In recent years, there has been renewed attention and interest in the use of herbal remedies globally. Nigeria is endowed with vast biodiversity of medicinal plants and a rich tradition of using them for curing various diseases and ailments. Many medicinal plants have been investigated for their putative anthelmintic efficacy, and a good number of them have also been found to be effective against various helminth parasites (Zahir et al, 2009; Tandon et al 2011; Yadav and Temjenmongla 2011, Yadav and Temjenmongla, 2012; Yadav and Tangpu 2012, Olusegun –Joseph et al., 2013, Williams et al., 2014, Mini et al., 2015,). Nevertheless, there are still a large number of medicinal plants in this country, which have long been used against helminth infections and without any scientific proof. This study investigated the anthelmintic efficacy of the thin layer chromatographic fraction of the leaf extracts of *Dalbergiella welwitschii* a traditional medicinal plant of Nigeria. From observation the thin layer chromatographic fractions of the water and methanol extracts of *Dalbergiella welwitschii* evaluated for its anthelmintic activity against earthworms (*Pheretima postuma*) and liver flukes (*Fasciola hepatica*) showed that the water extract eluted with methanol at Rf 0.86, methanol extract eluted with methanol at Rf 0.00 and methanol extract eluted with Butanol: Ethanol: Water at Rf 0.00 caused paralysis and death of liver flukes between 10:79 and 19:47 minutes, 12:86 and 21:29 minutes and 13:26 and 28:86 minutes respectively while the standard drug albendazole paralyzed and caused death of liver flukes in 46:66 and 129:31 minutes. The methanol extract eluted with Butanol: ethanol: Water at Rf 0.27 and water extract eluted with Butanol: ethanol: Water at Rf 0.37 caused paralysis and death of earthworms between 03:76 and 04:56 min and 05:47 and 06:21 min. The standard drug

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albendazole can used paralysis and death in 482:10 and 720:35 min as seen in tables 1 and 2. There was clear indication that the anthelmintic activity of the methanolic and water extracts of the thin layer chromatographic fractions of *Dalbergiella welwitschii* were more potent against liver flukes than on earthworms.

However, since the animals did not convulse before dying, it postulated that the extract did not kill the mice by some action on the nervous system (Ogwal-Okeng *et al.*, 2003, Ya'u et al., 2013). There were no morphological changes in the colour of the various organs of the treated animals compared to the control group in animals used for the subchronic toxicity test. There were slightly significant changes in the various organs weight especially liver and kidney in the animals that received the highest dose of the drug. The plasma protein showed no appreciable increase which suggested that the kidney did not compromise its function. The liver and heart release AST and ALT, and an increase in their plasma concentrations indicate hepatic and cardiac damage (Mythilypriya et al., 2007; Wasan et al., 2001). Since the levels of these two enzymes showed no appreciable increase in the treated animals, it implied that the medicinal plant had no effect on the liver and heart. The lack of significant alterations in the levels of ALT, AST, alkaline phosphates, glucose and creatinine which are good indicators of liver and kidney functions, suggests that sub-chronic administration of extract neither altered hepatocytes and kidneys of rats nor the normal metabolism of the animals. There were no significant alteration in the level of the haemotological parameters in all the treated groups compared to the control which indicates that the plant extract is safe for consumption.

CONCLUSION

It can be concluded that the thin layer chromatographic fractions of the extract of *Dalbergiella welwitschii* have profound anthelminthic activity against tested species of worm even better than the standard drug, did not provoke toxic effects on the animal's liver, hearts and kidney even at high doses. Water extract of the leaf of *Dalbergella welwitschii* is safe for consumption.

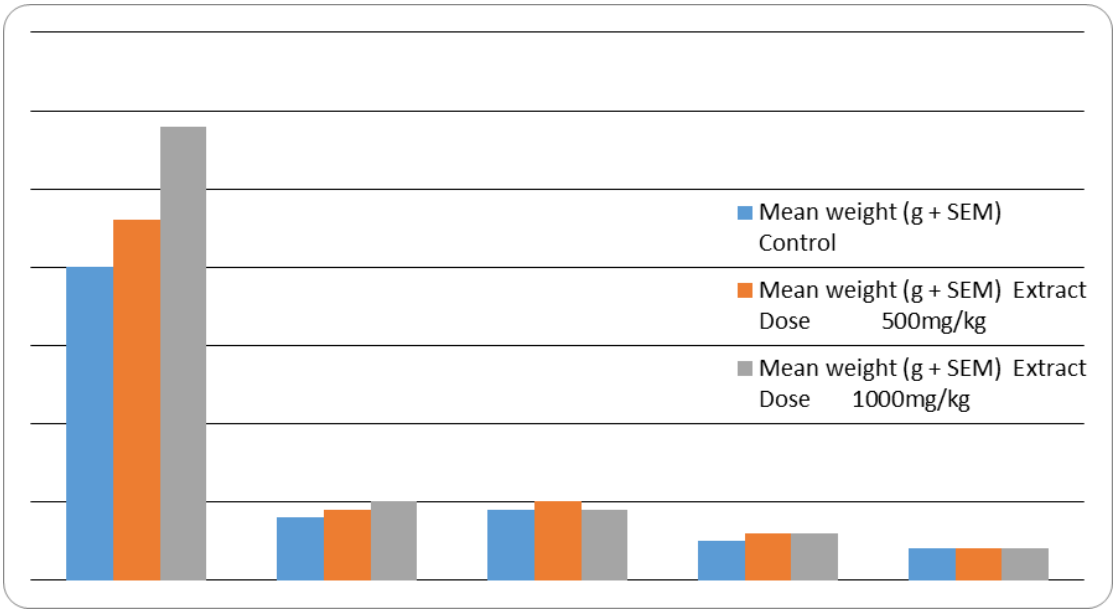

Figure 1: Mean organ weight of rats after 28 days treatment with aqueous leaf extract of *Dalbergiella welwitschii*

Table 3: Haematological parameters from rats after 28 days treatment with water extract of *Dalbergiella welwitschii*

Mean weight (g ± SEM)			
		Extract Dose	
Parameters	**Control**	**500mg/kg**	**1000mg/kg**
Haemogblobin (g/dl)	12.37 ± 0.40	14.16 ± 0.95	11.79 ± 0.20
PCV (%)	37.25 ± 1.18	42.66 ± 2.84	35.50 ± 0.50
WBC (mm^3)	6800 ± 746.9	683 ± 679.6	6100 ± 450.0
RBC (T/L)	5.33 ± 0.10	5.54 ± 0.40	4.68 ± 0.03
MCV (fl)	69.84 ± 1.03	77.14 ± 0.77	75.77 ± 0.50
MCH (pg)	23.20 ± 0.34	25.59 ± 0.20	25.17 ± 0.25
MCHC (%)	33.21 ± 0.06	33.18 ± 0.07	33.20± 0.11
Platelet (10, µl)	466.75 ± 16.4	407.33 ± 7.4	599.50 ± 5.5

Key: $p < 0.05$ (p means significant level

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Table 4: Biochemical parameters from rats after 28 days treatment with water extract of *Dalbergiella welwitschii*

		Extract Dose	
Parameters	**Control**	**500mg/kg**	**1000mg/kg**
AST (U/J)	163.9 ± 11.9	163.1 ± 2.4	189.2 ± 9.6
ALT (U) AL ALT(U/J)	51.57 ± 4.2	61.38 ± 0.6	47.91 ± 2.1
TP (g/L)	4.80 ± 0.06	4.82 ± 0.10	4.67± 0.19
Creatinine (mg/dl)	2.50 ± 0.34	1.97 ± 0.04	1.94 ± 0.52
Urea (mg/dl)	29.61 ± 0.83	38.21 ± 0.98	31.51 ± 0.34
ALP (U/I)	316.7 ± 23.1	231.8 ± 13.8	245.6 ± 19.3

Key: $p < 0.05$ (p means significant level)

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