

Full Length Research Paper

***In vitro* potential anthelmintic activity of bulbils of *Dioscorea bulbifera* L. on earthworms and liverflukes**

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Ethnobotanical information from Nigeria specifies the usage of *Dioscorea bulbifera* L. (Dioscoreaceae) in treatment of parasitic diseases in human and thus, could be of value in preventing the development of resistance to common synthetic anthelmintics. The present study was designed to evaluate the *in vitro* anthelmintic activity of methanolic extracts of the flesh and peel of the bulbils of *D. bulbifera*, on *Fasciola gigantica* and *Pheritima posthuma* at concentrations ranging from 10 to 100 mg/ml. Albendazole and normal saline were included in the assay as standard reference drug and control, respectively. Thin layer chromatography was used to screen the methanol extracts of the flesh and peel of the bulbils of *D. bulbifera* for important secondary metabolites in comparison with gallic acid and quercetin. The median lethal concentration values of the flesh and peel extracts of *D. bulbifera* were 39.67 and 30.40 mg/ml for earthworm and 61.73 and 41.79 mg/ml for liverfluke, respectively. The peel was more potent at 100 mg/ml, causing paralysis in 5.6 ± 0.51 min and death in 10 ± 0.45 min in earthworm. The findings from this study show that *D. bulbifera* possess *in vitro* anthelmintic compound worthy of further evaluation.

Key words: Albendazole, anthelmintic, *Dioscorea bulbifera*, *Fasciola gigantica*, *Pheritima posthuma*.

INTRODUCTION

The incidence of helminth infections is a global human health concern. Tropical regions of the world, particularly the Sub-Saharan African communities are among the worst hit by the diseases (Hotez et al., 2007). The majority of infections due to helminths causes enormous hazard to health, contributing to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia (Bundy, 1994). Parasitic diseases such as lymphatic filariasis, onchocerciasis and schistosomiasis cause ruthless morbidity affecting principally population in endemic areas (Tagbota and Townson, 2001). The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms which result in

weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs, 1986).

The use of synthetic anthelmintic drugs is part of important worm control strategy throughout the world. Ideally, an anthelmintic agent should have broad spectrum of action, high percentage cure with a single therapeutic dose, non-toxic to the host and should be cost effective (Ekeanyanwu and Etienjirhevwe, 2012). However, most of the common synthetic anthelmintics drugs available in the market are lacking in these requirement (Mali and Mahta, 2008). A contemporary challenge in the treatment of helminths diseases is the development of resistance by

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the parasites against conventional anthelmintics (Walter and Prichard, 1985; Geert and Dorny, 1995; Cole, 1997; Tagbota and Townson, 2001). Also, the high cost of modern anthelmintics has limited the effective control of these parasites and thus, has led to the screening of many medicinal plants for their anthelmintic activity in search for newer anthelmintic drugs (Akhtar et al., 2000; Abdel-Ghaffar et al., 2011; Klimpel et al., 2011; Tandon et al., 2011).

Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in humans such as lymphatic filariasis, onchocerciasis, schistosomiasis and could be of value in preventing the development of resistance (Hammond et al., 1997). Alkaloids, flavonoids, saponins and tannins have been demonstrated to possess anthelmintic activities (Ekeanyawu and Etienajirhevwe, 2012). In their findings, moderately high amount of tannin was reported in the aqueous, ethanol and methanol extracts of *Monodora myristica* and *Xylopi aethiopica* seeds and was possibly responsible for the significant anthelmintic activity. Chemically, tannins are polyphenolic compounds (Niezen et al., 1995). They also suggested that the presence of steroidal alkaloids oligosaccharides may have suppressed the transfer from the stomach to the small intestine which could diminish the availability of glucose to helminths together with its antioxidant effect which is capable of reducing the nitrate generation.

Alkaloids have been reported to act on the central nervous system of earthworms causing paralysis (Roy, 2010). Similarly, Nayak (2010) on the basis of phytochemical analysis and anthelmintic results of the crude extracts of *Hyptis suaveolens* suggested that phenolic content in the extracts produced similar effects as some synthetic phenolic anthelmintics like niclosamide, oxy-clozanide and bithionol which are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1994).

Dioscorea bulbifera, besides being important as an edible yam, is reported to have diuretic activity (Dhawan et al., 1997). Traces of diosgenin have also been detected in this species (Quigley, 1978). *D. bulbifera* is widely used in traditional Indian and Chinese medicine in the treatment of sore throat, gastric cancer and carcinoma of rectum and goiter (Gao et al., 2002). The various extracts of bulbils of the plant have been reported to be antihyperlipidemic (McKoy et al., 2003), antitumour (Gao et al., 2002), antioxidant (Jindal et al., 1969), analgesic and anti-inflammatory (Nguelefack et al., 2011), plasmid curing (Shririam, 2008) and antihyperglycemic (Ahmed et al., 2009). *D. bulbifera* is used in Bangladesh for the treatment of leprosy and tumours (Murray et al., 1984) and by the native people of the Western highlands of Cameroon for the treatment of pig cysticercosis. In Zimbabwe, the plant is used as an infusion applied on

cuts and sores, both for humans and animals while in Cameroon and Madagascar, the powdered bulbs are applied to abscesses, boils and wound infections (Cogney, 2002). In India, its bulbils are used to treat piles, dysentery, syphilis, ulcers, pain and inflammation (Gupta and Singh, 1989).

Ethnobotanical information obtained from the field of collection of *D. bulbifera* in Southwest Nigeria revealed that the powdered bulbil soaked in water is effective in reducing high blood pressure and that when it is roasted and eaten by farmers during the scarcity of other yam species, it destroys and expels microbes and parasites through the faeces. In view of the ethnomedicinal application of *D. bulbifera*, the present study was designed to evaluate the *in vitro* anthelmintic activity of methanol extracts of the flesh and peel of the bulbils of the plant.

MATERIALS AND METHODS

Plant collection and authentication

Bulbils of *D. bulbifera* were collected in Ibadan (7.40°N, 3.92°E; tropical wet and dry climate), Southwest, Nigeria in November, 2011. The authentication of the plant was done by Mr. O. A. Osiyemi at the Forest Herbarium Ibadan (FHI) where the voucher specimen FHI 109529 was deposited. Bulbils were separated into flesh and peel, dried under shade and ground into powder with the aid of an electric mill. The powder was stored in air-tight container at 4°C until use.

Plant extraction

Five hundred grams of powdered flesh and peel of the bulbils of *D. bulbifera* were macerated separately in a 2 L flask using redistilled methanol as solvent for a period of 72 h with intermittent stirring with a glass rod and filtered using filter paper (Whatman No. 1, Whatman® Schleicher and Schuell). The combined filtrates were concentrated using Rotavapor (Rotavapor R-210; Buchi Rotavapor) at a temperature of 40°C under reduced pressure. The extracts were stored at 4°C until needed for analysis.

Phytochemical screening

Standard phytochemical tests were carried out on the crude extract of the flesh and the peel of *D. bulbifera* to detect the presence or absence of carbohydrates, cholesterol, alkaloids, steroids/triterpenoids, tannins, flavonoids, anthraquinones, cardiac glycosides and saponins (Trease and Evans, 2002; Harborne, ; Sofowora, 2008).

Preliminary thin layer chromatography (TLC) screening

Thin layer chromatography (TLC) was further used to screen the methanol extracts of the peel and flesh of bulbils of *D. bulbifera* for important secondary metabolites using pre-coated TLC plates (Silica gel G 60 F₂₅₄ sheets 20 × 20 cm, 0.5 mm thickness, Merck). The extract and reference compounds (gallic acid and quercetin) were spotted on TLC plates and subsequently developed in suitable

solvent system containing ethyl acetate, methanol, ethanol and water in ratio 81: 11: 4: 8. The plates were dried, visualized in daylight and under ultraviolet (UV) lamp fluorescence at 254 and 365 nm before they were sprayed with 1% anisaldehyde in glacial acetic acid and 5% ferric chloride in 0.5 N HCl (Gage et al., 1951).

Test organisms

Liver flukes (*Fasciola gigantica*, 2.2 to 4.4 cm in length) were obtained from freshly slaughtered cattle in the Bodija abattoir, in Ibadan metropolis (7.40°N, 3.92°E). Earthworms (*Pheritima posthuma*, 5.5 to 12.5 cm in length) were collected from the water logged areas of Coca-Cola, Sango, Ibadan. Identification and authentication of worms were done by Dr. Soji Abiola of the Department of Veterinary Medicine, University of Ibadan. *P. posthuma* was used due to its anatomical and physiological resemblance with parasitic gastrointestinal nematodes in human being (Nirmal et al., 2007).

Anthelmintic bioassay

The anthelmintic study of the flesh and peel extracts against the selected worms (*P. posthuma* and *F. gigantica*) was conducted according to the method described by Ajaiyeoba et al. (2001) with slight modifications. Plant extract (10 g) was dissolved in saline water to make stock solution and different concentrations of (100, 70, 50, 20 and 10 mg/ml) were prepared for the anthelmintic assay. Albendazole (10 mg/ml) was included as reference drug, while saline water was included as control. Standard drug and extract solutions were freshly prepared before starting the experiment. For the evaluation of each plant extract, five worms (same type) were placed in a 9 cm Petri dish containing 25 ml solution of methanol crude extracts of plant in the tested concentrations. The plant extract was dispensed into the Petri-dish before introducing the worms. Observations were made for the time taken until paralysis and death of an individual worm. Mean time for paralysis (P in min) was taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D in min) were recorded after ascertaining that worms neither moved when shaken rigorously nor when dipped in warm water (50°C). The LC₅₀ was determined using a linear regression.

Determination of median lethal concentration (LC₅₀)

The worms were divided into twelve groups comprising five worms in each group. Groups 11 and 12 served as positive and negative control and received albendazole and saline water, respectively. Groups 1 to 5 of earthworms and groups 6 to 10 of liverflukes were treated with 10, 20, 50, 70 and 100 mg/ml dose of plant extract. Time of paralysis and death was observed for 24 h post-administration of the extract. From these observations, the median lethal concentration (LC₅₀) of the extract was calculated using Microsoft excel 2007.

Statistical analysis of result

All data were presented as mean ± S.E.M using Microsoft Excel 2007. Statistical analysis was performed using independent Student t-test (Graph Pad Prism version 6.0). Mean time of paralysis and death of worms were considered statistically significant at P<0.05.

RESULTS

The flesh and peel extracts of *D. bulbifera* showed significant anthelmintic activity at 100 mg/ml. The time of paralysis (P) and death (D) of the worms are presented in Figures 1 and 2. Anthelmintic activity was higher in the peel extract with LC₅₀ values of 30.40 and 41.79 mg/ml and coefficient of determination (R²) of 0.640 and 0.728 for earthworms and liverfluke, respectively (Figures 3 and 4). Earthworms were more sensitive to the peel extract of *D. bulbifera* as shown in Table 1. The peel extract produced paralysis in 5.6 min and death in 10 min while flesh extract showed paralysis in 8.4 min and death in 13.8 min at 100 mg/ml, when P and D for the reference drug (Albendazole) were 15.2 and 39.6 min, respectively at 10 mg/ml. Similarly, the peel extract exhibited significant anthelmintic properties with liverfluke. Liverflukes were paralyzed after 10.2 min and died after 15.81 min at 100 mg/ml whereas P and D for the reference drug were 28 and 50.6 min, respectively at 10 mg/ml. In this experiment, it took a longer time for the earthworms and liverflukes to die in both the flesh and the peel methanol extracts at 10 mg/ml. The preliminary phytochemical screening revealed the presence of phenolics (tannins, flavonoids), saponins as well as other secondary metabolites.

Thin layer chromatography analysis on pre-coated silica gel plates showed the presence of five to seven spots, representing different compounds in the methanol extracts of peel and flesh of *D. bulbifera* (Table 2). Phenolics and flavonoids were corroborated with the use of TLC which indicated their presence by showing spots on the plates having the same R_f values with reference compounds that is, gallic acid and quercetin. Spraying of the developed TLC plate with 5% ferric chloride in 0.5 N HCl showed a light blue colour and R_f value of 0.86 for both flesh and peel of *D. bulbifera* under UV lamp, 254 nm. This was comparable with the deep blue colour and R_f value of 0.83 observed with gallic acid under the same wavelength. The blue colour with almost similar R_f value indicate the presence of phenols in the extract.

DISCUSSION

Anthelmintic drugs are known to act by causing paralysis of worms or damaging cuticle, leading to partial digestion or to injection by immune mechanism. It also interferes with metabolism of worms since the metabolic requirements of these parasites vary greatly from one species to another (Aisawanya et al., 2010). Albendazole has been shown to affect worms by destroying the cytoskeletal structure of the worm thereby causing paralysis (Nikesh et al., 2011). Also, the predominant effect of piperazine citrate in worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis (Sini et al., 2011).

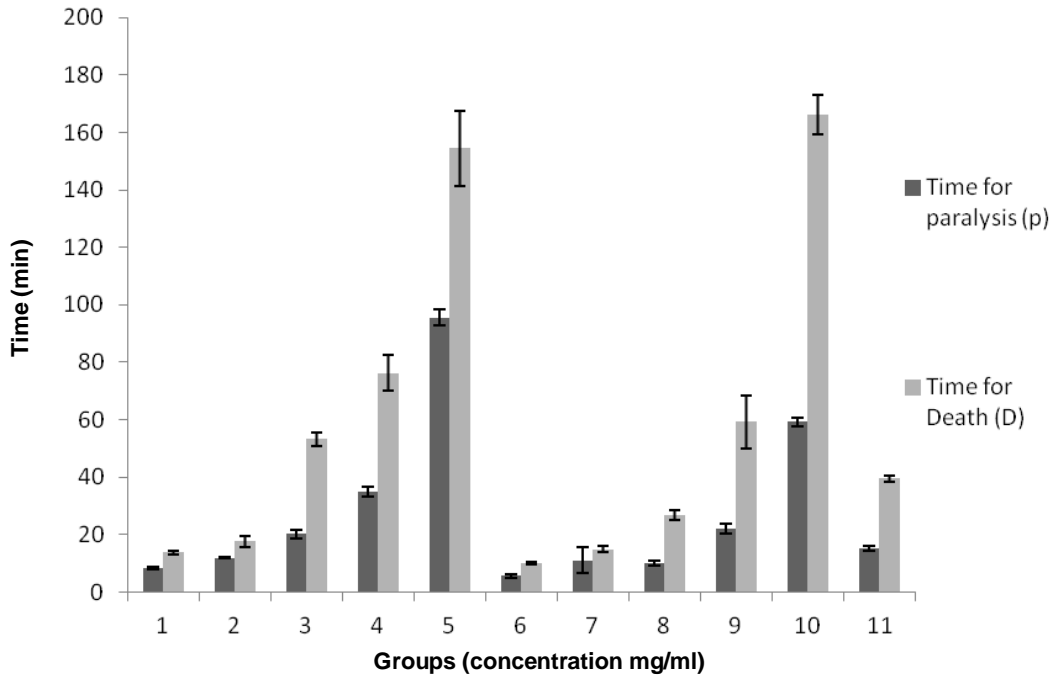


Figure 1. Anthelmintic Activity of Methanol Extracts of flesh and peel of bulbils of *Dioscorea bulbifera* using *Pheritima posthuma*. Groups (1 to 5) 100, 70, 50, 20 and 10 mg/ml of flesh of bulbils of *D. bulbifera*; Groups (6 to 10) 100, 70, 50, 20 and 10 mg/ml of peel of bulbils of *D. bulbifera* and Group (11) 10 mg/ml Albendazole as standard drugs.

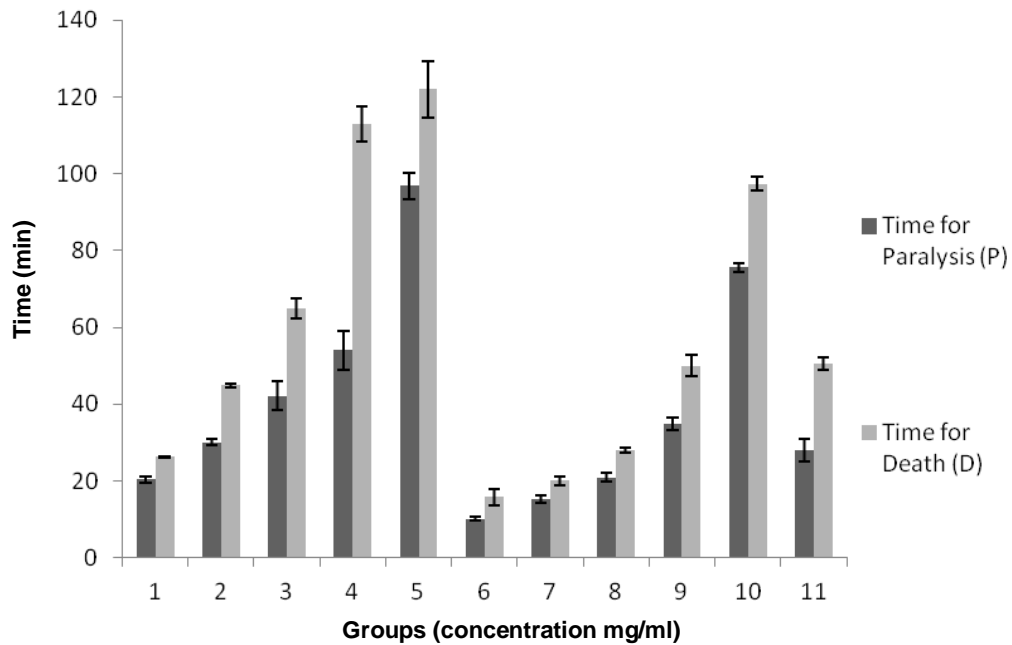


Figure 2. Anthelmintic Activity of methanol extracts of flesh and peel of bulbils of *Dioscorea bulbifera* using *Fasciola gigantica*. Groups (1 to 5) 100, 70, 50, 20 and 10 mg/ml of flesh of bulbils of *D. bulbifera*; Groups (6 to 10) 100, 70, 50, 20 and 10 mg/ml of peel of bulbils of *D. bulbifera* and Group (11) 10 mg/ml Albendazole as standard drugs.

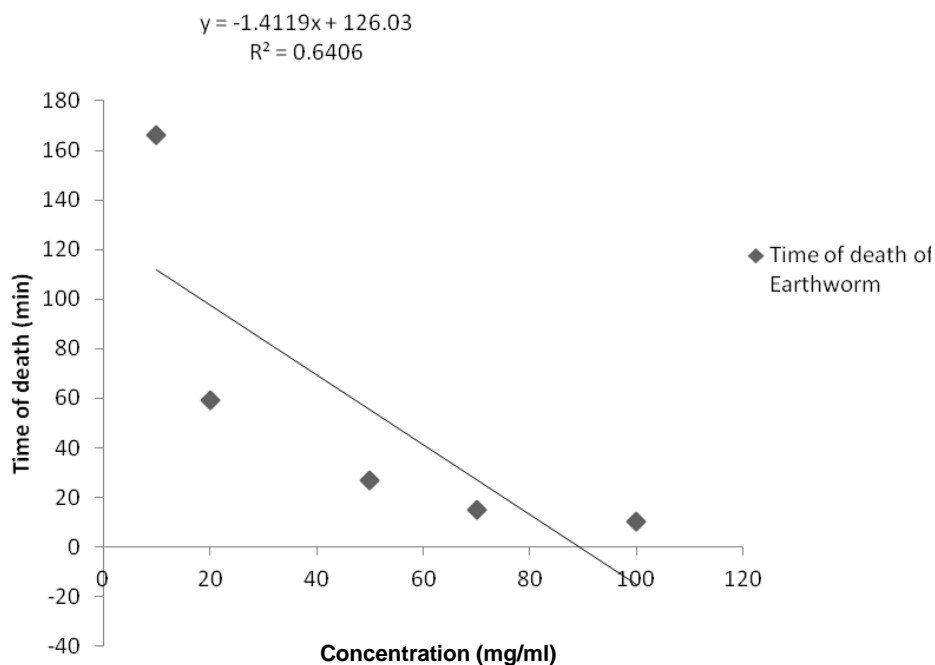


Figure 3. Regression equation of time of death of earthworms against concentration.

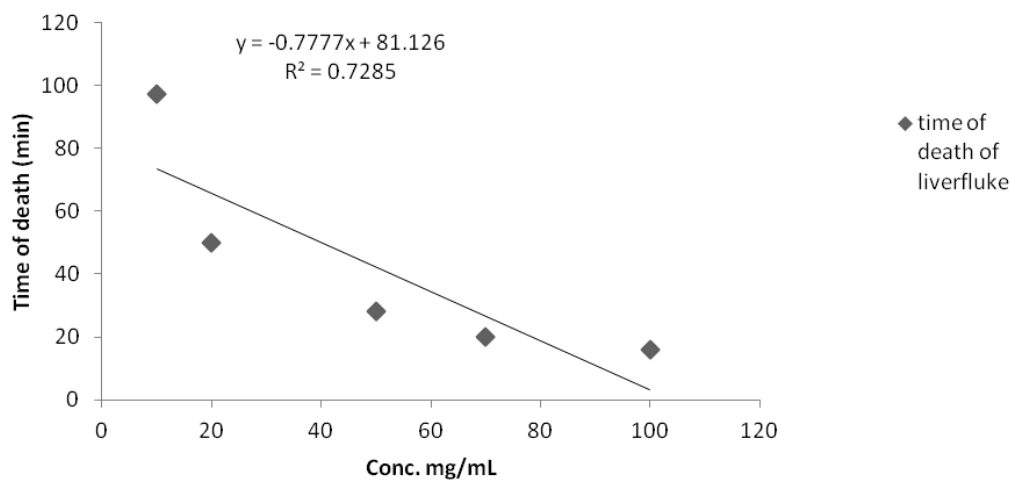


Figure 4. Regression equation of time of death of liverfluke against concentration.

Ekeanyanwu and Etienejirhevwe (2012) reported moderately high amount of tannin (phenolic compounds) in the aqueous, ethanol and methanol extracts of *Monodora myristica* and *Xylopia aethiopica*. They suggested that it could be responsible for the anthelmintic activity observed in their study in that it acts in a similar way to synthetic phenolic anthelmintic like niclosamide, oxcyclozanide and bithionol. These compounds are known to interfere with energy generation in helminth parasites

by uncoupling parasite specific reductase mediated oxidative phosphorylation reaction. Phenolic compounds were also reported to be responsible for anthelmintic activity in the evaluation of the root of *Raphanus sativus* (Devraj, 2011). Phenolic and tannin compounds show anthelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus lead to death of the worm (Kane, 2009). The extracts in the present study may have demonstrated this property resulting to subsequent death of

Table 1. Anthelmintic activity of methanol extracts of flesh and peel of the bulbils of *Dioscorea bulbifera*. *Pheritima posthuma* *Fasciola gigantica*.

Extract	Concentration (mg/ml)	P	D	P	D
Flesh of <i>D. bulbifera</i>	10	95.60±2.87*	154.40±13.16*	96.80±3.38*	122.0±7.37*
	20	35.00±1.70*	76.20±6.20*	54.00±4.94*	113.0±4.64*
	50	20.20±1.43	53.20±2.23*	42.20±3.83	64.80±2.63*
	70	12.00±0.32	17.60±1.75	30.10±0.71	45.00±0.50
	100	8.40±0.40	13.80±0.58	20.40±0.89	26.30±0.12
Peel of <i>D. bulbifera</i>	10	59.20±1.35*	166.20±6.89*	75.60±1.04*	97.30±1.82*
	20	22.20±1.74*	59.20±9.06	35.00±1.60*	50.00±2.86
Albendazole	50	10.20±0.86	26.80±1.88	21.00±1.14	28.00±0.71
	70	11.00±4.51	15.00±1.26	15.20±1.04	20.10±1.12
	100	5.60±0.51	10.00±0.45	10.20±0.50	15.81±2.13
	10	15.20±1.02	39.60±1.08	28.00±2.82	50.60±1.72

Time of Paralysis (P) and Death (D) of worms in minutes In the control (Normal saline treated) *P. posthuma* lived 36 h while *F. gigantica* lived 41/2 h. Values are expressed as mean ± SEM (n=5) *Means significantly different at P<0.05 compared with the Albendazole treated group in each column using independent student t- test

Table 2. Thin layer Chromatography of crude extract of the flesh and peel of bulbils of *Dioscorea bulbifera*.

Plant extract	Adsorbent	No. of Spot	R _f 1	R _f 2
Flesh	Silica gel	1	0.34	0.64
		2	0.59	0.69
		3	0.71	0.77
		4	0.79	0.86
		5	0.87	0.96
		6	0.91	-
Peel	Silica gel	1	0.31	0.64
		2	0.59	0.69
		3	0.69	0.77
		4	0.81	0.86
		5	0.84	0.89
		6	0.87	0.91
		7	0.91	0.96
Quercetin	Silica gel	1	0.82	0.94
Gallic acid	Silica gel	1	0.69	0.83

R_f 1: retardation factor 1 (spraying reagent: Anisaldehyde in sulphuric acid), R_f 2: retardation factor 2 (spraying reagent: Ferric chloride in 0.5N HCl); Solvent System (Ethyl acetate: methanol: ethanol: water; 81: 11: 4: 8).

the worms.

The main biological activity described for saponins based on recent research is their membrane permeability property. Possible actions of saponins include: changes in membrane permeability and pore formation, similar to

the activity of two conventional anthelmintic drugs-praziquantel and toltrazuril. The drugs have been reported to affect the permeability of the cell membrane of worms, causing vacuolization and disintegration of the teguments (Wang, 2010). The present evaluation of

anthelmintic properties of the bulbils of *D. bulbifera* gave data that showed both flesh and peel extracts to possess dose-dependent anthelmintic activity. However, the peel showed stronger anthelmintic activity at 100 mg/ml in destroying worms than that of the flesh. The anthelmintic activity of the peel extracts was more effective on the earthworms, with LC₅₀ at 30.40 mg/ml. The anthelmintic activity could be attributed to the presence of trace amount of phenolics and saponins in the plant which may have been responsible for the paralysis and subsequent death of the tested worms. Other medicinal plants where anthelmintic properties attributed to phenolics have been reported include: the root of *Baliospermum montanum* Muell (Euphorbiaceae) and the leaves of *Cassia tora* Linn. (Caesalpinaceae) (Molan et al., 2000; Mali and Wadekar, 2008; John et al., 2009).

Conclusion

This study suggests that flesh and the peel of the bulbils of *D. bulbifera* possess significant anthelmintic property. The present study is the first report on antihelmintic activity of *D. bulbifera* in Nigeria and the ethno-medicinal report of the plant as an anthelmintic drug is confirmed. Efforts shall be aimed at isolating and characterizing the compounds that are responsible for the anthelmintic activity and to establish the mechanism of action.

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