Arch. Bas. App. Med. 3 (2015) 29 - 36

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

In-vitro Antihelmintic and Kill Kinetics Activities of Stem Bark Extracts and Chromatographic Fractions of *Chrysophyllum albidum (*G. Don)

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Received: September 2014

Abstract

High resistance prevalent to most antihelmintic drugs has led to the search for alternative treatments. *Chrysophyllum albidum* G. Don a rain forest tree has folkloric anthelmintic claims. The anthelmintic properties of n-hexane, dichloromethane and methanol extracts and the chromatographic fractions of methanol extract were evaluated. Earthworms washed in distilled water (n=7) were placed in 9cm petri dishes of crude extract with six different concentrations (1,5,10,20,40,60mg/mL) and four concentrations (1, 5, 10 and 20mg/mL) of chromatographic fractions A,B and C. In the study two, liverfluke and tapeworms (n = 7) each washed in distilled water were placed in 9 cm petri dishes of crude methanol extracts of C. *albidum* (60mg/mL) or chromatographic fractions B or C (10mg/mL and 20mg/mL each). This test was conducted in duplicate. Gross motility and mortality studies were carried out as described by earlier method. Albendazole was used as a reference drug. Data is presented as Mean ± SEM, analysis conducted using two way ANOVA. Methanolic extract of C. *albidum* (MeCaB) showed the highest anti-helmintic activity at 60mg/mL comparable with Albendazole. Chromatographic fractions B and C (at 10 and 20mg/mL each) showed the highest antihelmintic activities with their time of death and paralysis comparable with Albendazole. Histological evaluation of the dead worms revealed that MeCaB and its' chromatographic fractions caused severe damages of epidermal cells. It can be concluded that methanol extract of *Chrysophyllum albidum* had greater anti-helmintic activity compared with other extracts and might be a good target plant source for antihelmintic drug development.

Keywords: Chrysophyllum albidum, antihelmintics, *in vitro* kill kinetics

INTRODUCTION

A large proportion of the world's population is affected by helminthic infection; it is common in human beings and poses a major threat to public health resulting in anaemia, malnutrition, eosinophilia and pneumonia (Ravindra and Mehta, 2008). Helminthes are the most common infectious agents of human gastrointestinal tract in developing countries and produce a global burden of disease that exceeds better known conditions, including malaria and tuberculosis (Peter *et al*., 2008).

Many helminthic infections occur in poverty-stricken and developing countries with warm, moist environments and poor sanitary conditions. Helminths can live in humans and animals. A few of helminthic infections often lead to death with most of them causing severe physical impairment. Most children in developing nations are vulnerable to helminth infections (Peter *et al*., 2007). The World Health Organisation reports a 35% infection rate for roundworms, a common parasitic worm (Krishnamuti, 2003; Perry *et al*., 2002).

Across Africa, malaria parasites and helminths occur in the same regions (Brooker *et al*., 2007) with School age children bearing the most burden of these diseases. Often than none, these infections do not occur singly but in a multiple way leading to even severe consequences (Brooker *et al*, 2007). In that study, helminthic infections were reported to increase susceptibility to malarial infection. Co-infection between these infections or diseases (malaria and helminthes) are accompanied by anaemia which has been documented in several countries of Africa thus requiring urgent need for better control measures. In addition to these susceptibility to co-infection, the presence of these worms in the body are believed to modulate the immune system of the host (Else *et al*., 1991*;* Khan *et al*., 2002).

Chemotherapeutic agents (anthelmintics) cause either total paralysis or death of worms (Afroz *et al*., 2013) in the gastrointestinal tract or systemically. There are two main important factors used in identifying a potent anthelmintic, the drug or plant must be able to (i) be absorbed or penetrate the cuticle of the (parasitic) worm or (ii) enter its alimentary tract (Dhumati *et al*., 2012). The indiscriminate extensive use and sole reliance on anthelmintics is under beginning to poses serious threat due to rapid and widespread emergence of anthelmintic resistant strains (of parasites) throughout the

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world including Nigeria and India (Singh *et al*., 2002). Moreover, some of these anti-helminths drugs are unaffordable, inaccessible or inadequately available to the easily infected poor people and unprotected farmers of most developing countries. Therefore alternative therapy such as herbal medicines has been resorted to by this lower class of people. These herbal medicines have been used over generations and their as a result, their possible different modes of action and potential clinical significance has been tapped into in drug discovery and development (Afroz *et al*., 2013).

For instance, use of plants such as *Ocimum sanctum, Pilliostigma thonningii, Melia azedarach* and plant derived preparations from herbal therapy show great promise as source of easily available and effective anti-helmitic agents. (Yoganandan *et al*., 2009; Dornetshuber *et al*., 2009; Gbolade *et al*., 2008; Enwerem *et al*., 2001).

Chrysophyllum albidum G. Don (African star apple) is a tropical rain forest fruit tree with an array of biological activities. The African star apple has been found to contain the highest content of ascorbic acid with 1000-3300 mg of ascorbic acid per 100gm of edible fruit or about 100 times that of oranges as well as 10 times that of guava or cashew (Asenjo *et al*., *1946*). It has also been reported to be an excellent source of vitamins, irons, flavours to diet and raw materials to some manufacturing industries (Adisa *et al*., 2000; Bada, 1997, Okafor and Fernandes *et al*., 1987).

The tree bark is used as a remedy for yellow fever and malaria, with the leaves used as emollients and for skin eruptions, diarrhea and stomach-ache treatment which might be as a result of infections and inflammatory reactions (Adewusi *et al*., 1997). Cotyledons from the seeds of *Chrysophyllum albidum* are processed into ointments which are used for the treatment of vaginal and dermatological infections in Western Nigeria. The plant leaves has been found to possess anti-hyperglycemic and hypo-lipidemic effects (Olorunisola *et al*., 2008). Various plant parts of *Chrysophyllum albidum* has also been found to exhibit antiplasmodial (Adewoye *et al*., 2010), antimicrobial (Adewoye *et al*., 2011), anti-nociceptive, anti-inflammatory and antioxidant activities (Idowu *et al*., 2006). The leaves and stem have been shown to contain β-amyrin acetate (Lopez *et al*.,1983), gentisic acid (Griffiths *et al*.,1959) and alkaloids (Smolenski *et al*.,1975; Delande *et al*.,1979), Eleagnine is an alkaloid which has been isolated from *Chrysophyllum albidum* seed cotyledon (Idowu *et al*.,2006). The isoprene content of the latex has been characterized and found to consist of polyisoprene (Nwadinigwe *et al*., 1988). The milled dried seeds are also been used in folk medicine for the treatment of intestinal worms (Houessou *et al*., 2012). The phytochemical profile shows presence of an array of biologically active substances including alkaloids, tannins, saponnin, phenol and flavonoids (Adewoye *et al*., 2011*;* Okoli *et al*., 2010). This array of biologically active substances might be responsible for the exhibited medicinal attributes to the various plant parts.

There is still an increasing search for safe, cheap, more promising effective anti-helmintic plants with antiplasmodial activities. *Chrysophyllum albidum* bark has been scientifically proven to possess a number of folkloric biological activities amongst which are antiplasmodial (Adewoye *et al*., 2010) and erythropoietic activities (Adewoye *et al*., 2012) but there is a dearth of information on its anti-helmintic properties and / or probable mechanism of action. Hence in this work, the bark extracts and chromatographic fractions of the plant bark have been evaluated for its possible anthelmintic activity.

MATERIALS AND METHODS

Plant Materials, Collection and Authentication: Fresh *C. albidum* barks were collected from its natural habitat between the month of January to April 2013 at Igbo Owe farm at Moniya, Akinyele Local Government Area of Oyo State, South-Western Nigeria. Identification of the plant was done at the Forest Herbarium Institute (FHI), Ibadan, Nigeria, where a voucher specimen (FH1 107514) was deposited. The barks were dusted of sand grains and insects, dried at room temperature for about three to four weeks before it was milled into powdery form. This was stored in air-tight containers until use.

Preparation of The Plant Extracts: The powdered bark of *C. albidum* (1.5kg) was well macerated in 2.5L of 100 % n-hexane for 72 hours with occasional shaking after which it was filtered (Whatman filter paper No.1) and the filtrate concentrated *in vacuo* at 40 ^{o}C.to dryness to give the n-hexane extract. The marc was spread out evenly, turned frequently and allowed to dry properly for 24 hrs. To the dried marc was added 2.5L of dichloromethane and allowed to stand for 72hrs with intermittent shaking before filtration and concentration to dryness to give the dichloromethane extract. The marc was dried for a day and again extracted with 2.5L of methanol for 72hrs and similarly treated to give the methanol extract. The methanol extract obtained was a solid (26g) chocolate brown residue (65% yield) and was further purified using the column chromatographic technique.

Column Chromatographic Fractionation: A portion of the methanol extract (50g) was dissolved in methanol and loaded on Silica gel (60-200 mesh size) in the column with dimension of 5cm in width and 30.5 cm in length. Gradient elution with n-hexane / ethylacetate / methanol was performed to obtain pooled fractions (A, B, and C) using thin layer chromatographic profiles of the each 50 mL eluted fractions.

Phytochemical Screening: The plant extracts were screened for alkaloids, steroids, saponin, tannin, reducing sugar and other glycosides using standard laboratory procedures (Harbone,1991). LC/DAD profiles of the methanol extract and chromatographic fractions were collected with an Alliance 2690 Separations Module (Waters) and PDA 996 (Waters) on a Symmetry column (C_{18}, 5 µm, 150 mm x 10 mm,) and Symmetry guard column (C_{18}, 5 µm, 20 mm x 10mm) using gradient elution (solvent A, 0.05% TFA in water; Solvent B, methanol). The mobile phase mixture consisted of 100% solvent A over 10 mins, changing to solvent A : solvent B (75:25) at 25 minutes and maintained at this composition to 45 minutes.

Anthelmintic Study

Worm collection: *Pheretima posthuma* (earthworm; of length 10 cm to 12 cm and weight 1.10g to 1.40g) were collected from the Awba dam within the campus of the University of Ibadan. The worms were transferred into a glass jar with some quantity of habitat soil from which they were taken for the experiment. The earthworms were rinsed off the debris with distilled water before they were used for the experiment.

In another study, live helminths were obtained from Kaara abattoir located at Bodija market, Ibadan, Oyo State, Nigeria. The helminths were preserved in a suitable environment to enable their survival outside their host. Tapeworms (*Taenia*

spp.; weighing between 3.67g to 4.00g) were preserved in a glass jar also containing intestinal contents from its host while Liver flukes (*Fasciola hepatic*a; weighing between 0.66g to 0.98g) were also preserved in a glass container containing blood and a portion of a liver removed from the host affected organ. These precautions were carried out in order to sustain and enhance the survival rate of the organism because an interruption in the absorption of nutrients can lead to death of the parasites before getting to the laboratory due to their inability to store nutrients. These samples were identified by a parasitologist from the Department of Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

Anti-helminthic assay (**Gross motility and mortality):** In the first study, live parasitic worms (n = 7) were cleansed of intestinal fluid by gently rinsing them into distilled water before weighing and transferring into petri dishes (in duplicates) containing different concentrations (1,5,10,20,40,60 mg/mL) made in distilled water of *Chrysophyllum albidum* fractions (n-Hexane, Dichloromethane, Methanol), Column fractions (A, B and C) and Albendazole.

In the second study, five Flatworms or Liverfluke were placed in a petri dish containing 60 mg/mL of C. *albidum* plant extract, 10 mg/mL or 20 mg/mL of chromatographic Fractions B or C or Albendazole. This test was also conducted in duplicate for the worm types.

The method was carried out as described by Ajaiyeoba *et al.*, 2001. No movement of the worm when pricked with a pin indicates total paralysis. Time of death was also ascertained when worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Albendazole (10 mg/mL and 20 mg/mL) was used as a reference drug due to its usage as a broad spectrum medicine. Some specimens were also placed in distilled water which served as control.

Once these helminthes were certified dead, the worm samples were preserved using bouin fluid as a fixing and preserving medium before taking them to the histology laboratory in the Faculty of Veterinary Medicine, University of Ibadan for histological preparations. Bouin fluid was chosen as a fixative in order to increase the size of the specimen for better viewing.

Statistical analysis

Data is presented as Mean ±SEM (n=5).The analysis between doses in treatment groups and the control was conducted by two way ANOVA and values were significant at p = 0.05.

RESULTS

LC/DAD profile of Chromatographic Fractions (CFrA, B and C).and crude Methanol extract (MeCaB,):

CFrA result revealed the presence of very high flavonoid peaks compared with CFr B or C (Fig. 1). CFrB and CFrC Results revealed the presence of much tannin peaks compared with Flavonoids. Shown Peaks are F (flavonoid) and T (tannins)

Effects of *Chrysophyllum albidum* extracts and fractions on Total Paralysis (Minutes) of adult Earthworms (*Pheretima posthuma*);

The *MeCaB* exhibited a significant decrease in the time taken for total paralysis of the earthworms from 1mg/mL to 60mg/mL when compared with N-hexane and Dichloromethane extracts. There was however no significant difference between the *MeCaB* and Albendazole at 10mg/mL to 20mg/mL in the course of this experiment (Table 1).

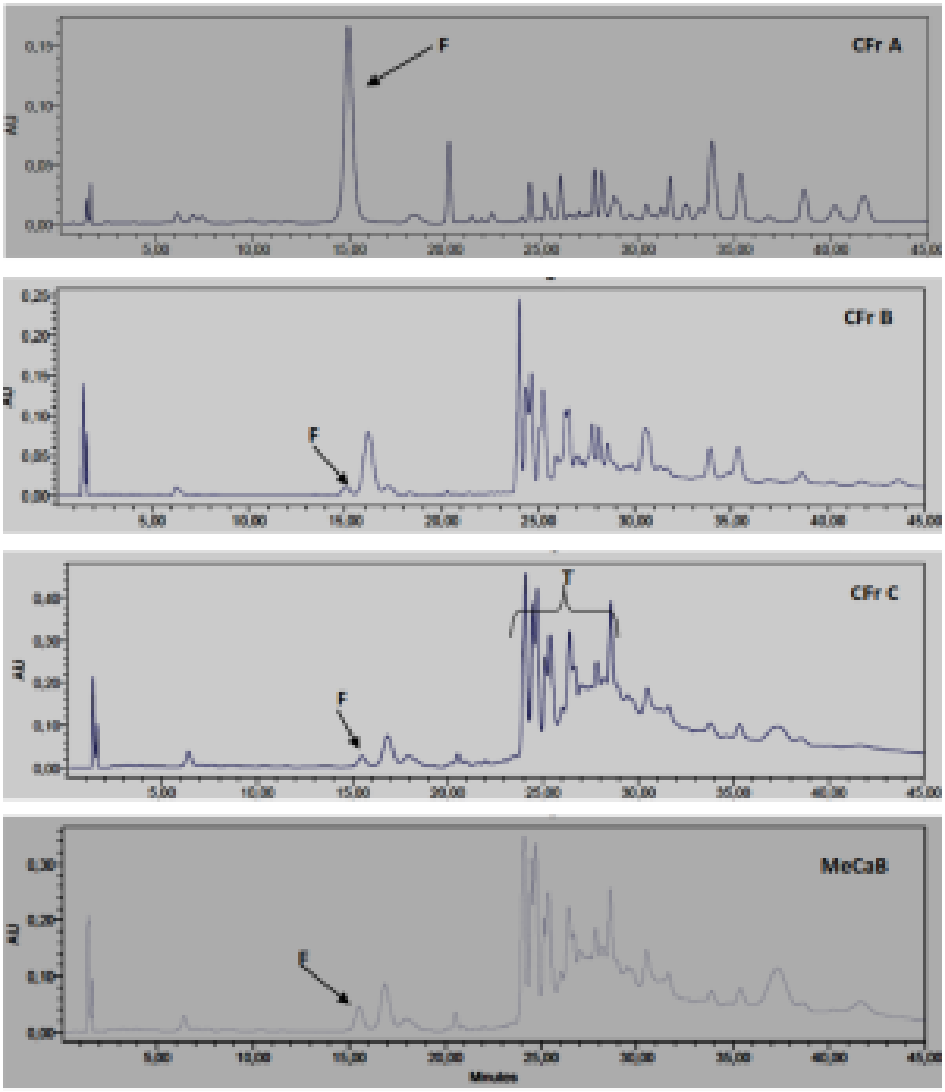

Figure 1:
LC/DAD profile of Chromatographic Fractions (CFrA, B and C).and crude Methanol extract (MeCaB,). Shown Peaks are F (flavonoid) and T (tannins)

Table 1:
Antihelmintic Activities Of *Chrysophyllum albidum* Extracts And Fractions On Total Paralysis (Minutes) of adult Earthworms

Extracts/doses	1mg/ml	5mg/ml	10mg/ml	20mg/ml	40mg/ml	60mg/ml
N-hexane	1339.00± 61.78	1291.00± 67.68	599.00± 63.64	546.60± 8.69	369.30± 2.54	52.86 ±9.59
Dichloromethane	1291.00± 67.68	188.00±0.00 [a]	145.00± 0.00 [a]	114.00± 0.00 [a]	48.47 ±0.09 [a]	38.39 ± 0.09
Methanol	87.86 ± 1.84[a,b]	73.00 ±0.00[a,b]	35.00 ± 0.00[a,b]	29.00 ±0.00[a,b]	9.14 ± 0.83[a,b]	4.71 ± 0.36[a,b]
Albendazol	ND	ND	18.03 ± 0.02[a,b]	10.33 ± 0.02[a,b]	ND	ND
Fraction A	ND	ND	ND	8.00± 0.00[a,b,d]	ND	ND
Fraction B	280.70± 64.25[a,b]	46.71 ± 6.26[a,b]	26.86 ±2.03[a,b]	13.57 ± 0.43[a,b,c]	ND	ND
Fraction C	60.00 ± 0.00[a,b]	60.00 ± 1.41[a,b,c]	31.57 ± 0.00[a,b]	16.00 ± 0.00[a,b]	ND	ND

Values are expressed as Mean ± SEM and[a,b,c,d and e] indicates significance ($p \leq 0.05$) when compared with n-hexane, Dichloromethane, Methanol, Albendazol and Chromatographic Fraction A respectively. 'ND' indicates that there was no experiment at the doses

Table 2:
Antihelmintic Activities of *Chrysophyllum albidum* extracts and Fractions on Time of Death (Minutes) of Adult

Extracts/doses	1mg/ml	5mg/ml	10mg/ml	20mg/ml	40mg/ml	60mg/ml
N-hexane	1414.00 ± 40.41	1000.00 ± 0.00	754.30 ± 68.69	830.00 ± 0.00	561.00 ± 0.00	405.70 ± 16.60
Dichloromethane	605.00 ± 0.00	298.00 ± 0.00 [a]	238.00 ± 0.00 [a]	203.00 ± 0.00 [a]	124.70 ± 0.84 [a]	64.71 ± 0.36 [a]
Methanol	306.40 ± 3.03	73.00 ± 0.00[a,b]	56.00 ± 0.00[a,b]	49.00 ± 0.00[a,b]	14.14 ±0.77[a,b]	11.57 ± 0.53[a,b]
Albendazol	ND	ND	26.26 ± 0.02[a,b]	16.29 ± 0.06[a,b]	ND	ND
Fraction A	ND	ND	ND	64.86 ± 21.62[a,b,d]	ND	ND
Fraction B	429.30 ± 54.55[a,b]	62.43 ± 6.87[a,b]	46.29 ± 6.47[a,b]	27.71 ± 0.61[a,b,c]	ND	ND
Fraction C	253.00 ± 42.97[a,b]	86.29 ± 8.64[a,b,c]	49.71 ± 0.61[a,b]	23.29 ± 2.22[a,b,e]	ND	ND

Values are expressed as Mean ± SEM and[a,b,c,d and e] indicates significance ($p \leq 0.05$) when compared with N-hexane, Dichloromethane, Methanol, Albendazol and Chromatographic Fraction A respectively. 'ND' indicates that there was no experiment at the doses

Table 3:
Antihelminthic activities of *Chrysophyllum albidum* extracts and fractions on the time (minutes) of death of adult liverflukes (*fasciola hepatic*a).

Extracts/ Fractions/ Drug	10mg/ml	20mg/ml	60mg/ml
Methanol	ND	ND	3.60 ± 0.24
Albendazole	3.60 ± 0.24	3.40 ± 0.24	ND
Fraction B	4.35 ± 0.64	6.20 ± 0.48	ND
Fraction C	4.35 ± 0.64	4.40 ± 0.40	ND

Liver flukes in distilled water acting as control took a longer mean time of death; Values are expressed as Mean ± SEM. 'ND' indicates that there was no experiment at the doses

Table 4:
Antihelminthic activities of *Chrysophyllum albidum* extracts and fractions on the time (minutes) of death of adult tapeworms

Extracts/ Fractions	10mg/ml	20mg/ml	60mg/ml
Methanol	ND	ND	7.20 ± 0.80 [ab]
Albendazol	14.00 ± 0.44	11.80 ± 0.91	ND
Fraction B	9.40 ± 0.24[a]	8.00 ± 0.83[ab]	ND
Fraction C	5.00 ± 0.63 [ab]	7.66 ± 1.05[ab]	ND

Values are expressed as Mean ± SEM [a,b] indicating significance $p \leq 0.05$ when compared with Albendazole (Alb10 mg/mL and 20 mg/mL) respectively. 'ND' indicates that there was no experiment at the doses

A significant decrease in the time of paralysis was observed in the CFrA compared with N-Hexane, Dichloromethane and Albendazole at 20mg/m (Table 1).

The CFr B and C exhibited a significant decrease in the time of paralysis of the earthworm when compared with N-Hexane and Dichloromethane at 1mg/mL to 60mg/m (Table 1).

Effects of *Chrysophyllum albidum* extracts and fractions on Time of Death (minutes) of adult earthworms (*Pheretima posthuma*);

The *MeCaB* showed a significantly reduced time of death of the earthworm at 1mg/mL to 60mg/mL when compared with N-hexane and Dichloromethane extracts (Table 2)

The CFrB and C showed a significantly decreased time of death when compared with N-Hexane, Dichloromethane and MeCaB extract (Table 2)

However, there was no significant difference in the time of death of earthworm immersed in Albendazol when compared with CFrB and C at 20mg/mL.

Effects of *Chrysophyllum albidum* extracts and fractions on the time (minutes) of death of adult liverflukes (*Fasciola hepatic*a);

Results shown in Table 3 shows that there was no significant difference in the time of death of the Liverfluke when compared with all the groups.

Effects of *Chrysophyllum albidum* extracts and fractions on the time (minutes) of death of adult tapeworms *(Taenia spp.)*;

The plant treated (*MeCaB*; CFr B and C) showed a significantly decreased time of death when compared with Albendazole and distilled water (Table 4).

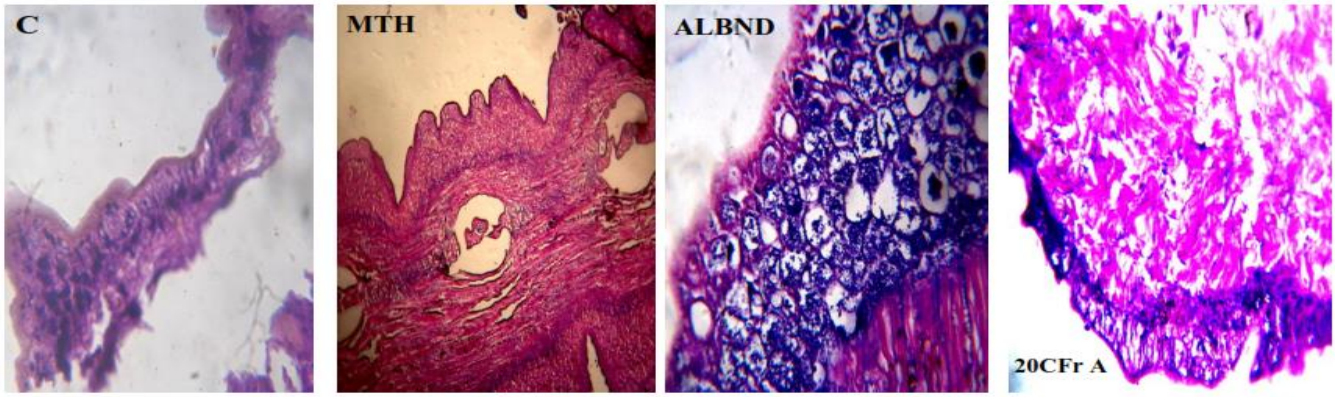

PLATE 1
Photomicrograph of sections from earthworm (h&e stain , mag x 400) showing C (CONTROL):- Normal skin with intact cuticle, epidermal cells and underlying muscle fibres. MTH (methanol extract 60mg/ml):- Absence or Loss or discontinuity of cuticle and moderate swelling (ballooning degeneration of epidermal. ALBND (Albendazol):-marked ballooning degeneration of hyperplastic epidermal cells, with intact muscular layers. 20CFrA (20 mg/mL Chromatographic fraction A):- A few foci of discontinuous cuticle, epidermal cells with karyorrhectic nuclei. There is multiple foci of epidermal necrosis/excoriations and moderate degeneration of underlying muscle fibers.

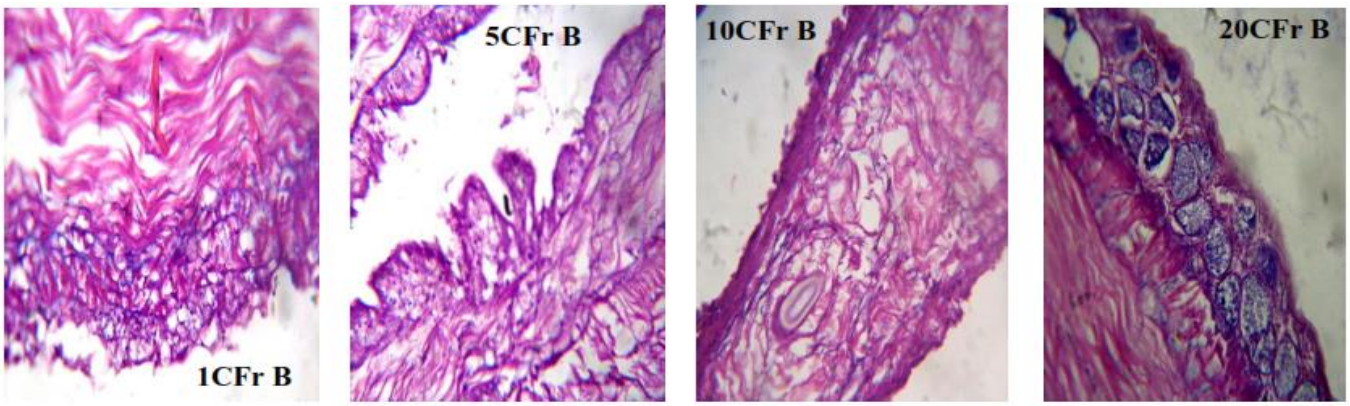

PLATE 2
Photomicrograph of sections from earthworm (h&e stain , mag x 400) showing 1CFrB(Chromatographic fraction B, 1 mg/mL):- Severe sloughing off or loss of epidermal cells and is markedly reduced in height. There are a few degenerate muscle fibers. 5FrB (5 mg/mL Chromatographic Fraction B):- There is hyperplastic epidermis and absence of thick cuticle. 10FrB (10 mg/mL Chromatographic fraction B):- Severe loss of the epidermal cells, disoriented muscle fibers and absence of cuticle. There is an abrupt junction with severe necrosis of epidermal cells and degenerate muscle fibers (appearing bluish) on the left and epidermal cells undergoing severe ballooning degeneration are present on the right. 20FrB (20 mg/mL Chromatographic fraction B):- The cuticle is absent and there is marked vacuolar / ballooning degeneration.

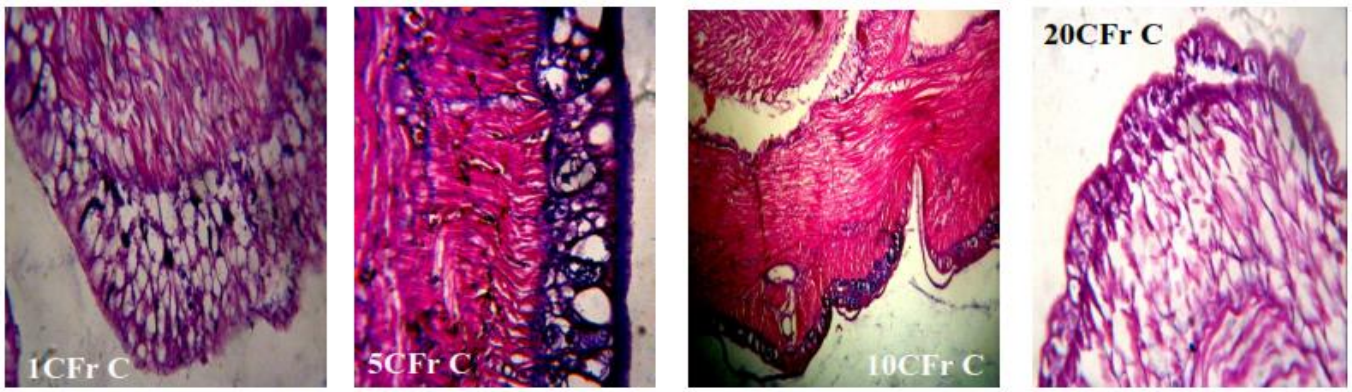

PLATE 3
Photomicrograph of sections from earthworm (h&e stain , mag x 400) showing 1CFr C (Chromatographic Fraction C, 1 mg/mL):- There is no cuticle and there is mild hyperplasia of the epidermis. 5CFr C (5 mg/mL Chromatographic fraction C):- There is mild vacuolar degeneration of the epidermal cells. 10CFr C (10 mg/mL Chromatographic Fraction C):- There is hyperplastic epidermis and the cuticle is absent. 20CFr C (20 mg/mL Chromatographic Fraction C):- The cuticle is present and there is balloon degeneration of epidermal cells, the muscle fibers are degenerated and sparse

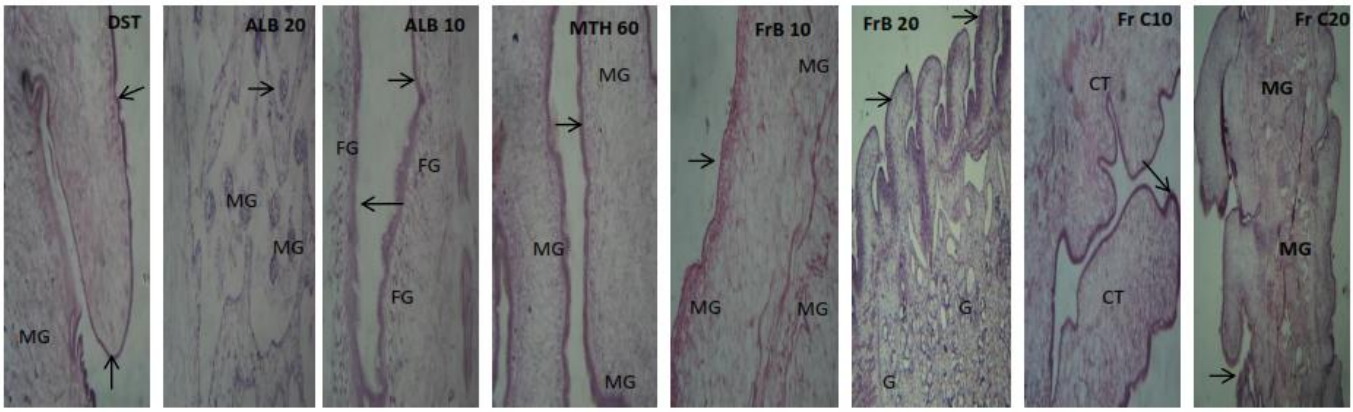

PLATE 4:
Photomicrographs of tapeworm sections (mag x 100) showing dst (control):- A few locally extensive foci of sloughing off of cilia (black arrows) with intact cuticle; well populated male gametes (MG), ALB (Albenadazole) 20mg/mL: Focus of disruption/swelling of cuticle (black arrow); other parts of the cuticle have intact cilia; numerous male gametes (MG); adequate amounts of underlying connective tissue ALB 10mg/mL: Multiple foci of slouging off of cilia (black arrow) and swelling of underlying cuticle; sparse amounts of loose connective tissue; depleted female gametes (FG) (eggs: dark blue structures in some cyst-like cavity) MTH (methanolic extract of C.*albidum*) 60 mg/mL: Severely depleted male gametes (MG); moderate overgrowth of cilia (black arrow); FrB 10mg/mL: Moderate extensive loss of cilia (black arrow); moderately depleted male gametes (MG); Fr B20 mg/mL: Fairly intact cuticle with a few foci of loss of cilia; intact loose connective tissue; numerous closely packed cavities that are depleted of gametes (G); Fr C10 mg/mL: Essentially normal covering cuticle except for a focus of loss of cilia (black arrow); variable amounts of loose connective tissue (CT); Fr C20 mg/mL: Covering cuticle with alternating areas of extensive loss of cilia (black arrow) and over-growth of cilia with moderately depleted reproductive cells (male gametes, MG).

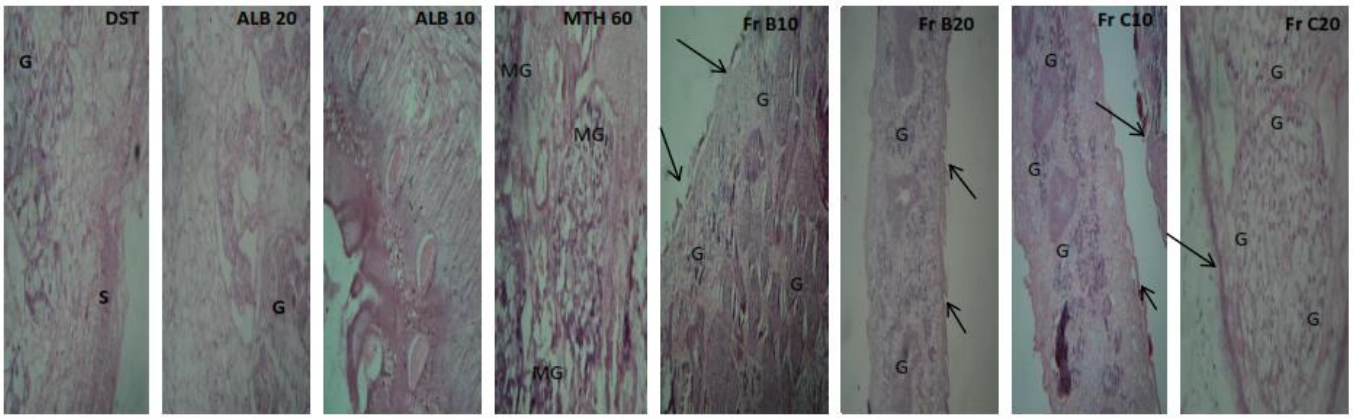

PLATE 5:
Photomicrographs of liverfluke sections (mag x 100) showing DST (control) : Severe extensive swelling of the cuticle (S) (cuticle is thickened); there are a few depressed foci of loss of cuticle with exposure of underlying connective tissue; moderate amounts of closely-packed cavities with sparse amounts of gametes (G); ALB 20: Intact cuticle; extensive loss of cilia; intact underlying connective tissue; moderate amounts of gametes (G); ALB (Albendazole) 10 mg/mL: there is however dense accumulation of connective tissue just under the cuticle as well as the presence of some discrete, well-circumscribed, oval, eosinophilic structures [possible necrotic tissue?]; few far-apart cavities containing little amounts of gametes (G); MTH (Methanolic extract of C.*albidum*) 60 mg/mL: Severely disrupted cuticle (black arrow) with absence of cilia, Numerous and adequate male gametes (MG); severe swelling of the cuticle; no normal even-height appearance; Fr B10 mg/mL: Severe, extensive disorganization of the cuticle (black arrow) with sloughing off as well as exposure of underlying connective tissue to the exterior; moderate amounts of gametes (G); Fr B20 mg/mL: Severe swelling and loss of cuticle (black arrow), thereby giving the helminth's surface a rough appearance; exposure of underlying loose connective tissue is frequent; moderate amounts of underlying connective tissue; numerous closely packed cavities containing little amounts of gametes (G); Fr C10 mg/mL: Multiple foci of severe sloughing of cilia and cuticle (black arrow) with exposure of underlying connective tissue to the exterior; fairly adequate gametes (G); Fr C20 mg/mL: A few foci of out-pouching and sloughing off of the cuticle (black arrow); at some foci; there is overgrowth of the cilia; there are numerous, closely-packed cavities containing gametes (G).

DISCUSSION

In the first study, the different extracts (N-hexane, Dichloromethane and Methanol) of *Chrysophyllum albidum* were screened for possible antihelmintic activities. The three extracts showed a dose-dependent anthelminthic activity on the earthworm. Results obtained showed that methanol extract (60 mg/mL) had the highest antihelmintic activity compared with other extracts (N-Hexane and Dichloromethane) of *C. albidum* and was in comparable range with Albendazole, the reference drug used

The methanolic extract of C. *albidum* was further fractionated by column chromatography with the aim of obtaining its active principle which is acting as an antihelminth. This purification afforded three (3) pooled fractions (CFrA, B and C) which were also evaluated for their antihelmintic activities with albendazole as the reference drug. Results showed that there was no significant difference

between this control drug and the Fractions B and C both in the time of paralysis or death.

In the second study, 60 mg/mL of C.*albidum*, Fractions B and C gave a faster rate of kill of the tapeworm parasites than albendazole, with 60 mg/mL of C.*albidum* and fraction C showing a higher vermicidal potential. Histological evaluation of the dead tape-worms and liverflukes from Study B revealed that the methanolic extract of *C.albidum* and its chromatographic fractions caused loss/discontinuity of cuticle, degenerated muscle fibre, severe sloughing off and or loss of epidermal cells. The methanol extracts and chromatographic fractions of *C. albidum* must have presumably penetrated the cuticle of the worm and interfered with its metabolism and store of energy causing death of the worms which is in line with observations of Dhumati *et al.*, 2012.

Dhunmati *et al.*, 2012 explained in their study that an effective anthelmintic must be able to penetrate the cuticle of most worms or gain access to its alimentary tract hence interfering with its' metabolism and requirement for survival within its host. It could also act by causing total or partial paralysis, or damaging of the worm's cuticle, leading to partial digestion or its' rejection by the host immune system.

The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristaltic action of the gastrointestinal tract. Albendazole exerts its' flaccid action on worms by increasing chloride ion conductance of worm muscle membrane thus producing hyperpolarisation which ultimately leads to reduced excitability and muscle relaxation. Albendazole has also been reported to cause paralysis in worm by disrupting the microfilaments, microtubules and β-tubulins component of their cytoskeletal structure (Nikesh *et al.*, 2011).

Earlier researchers have attributed the antihelmintic activities of medicinal plant parts to certain phytochemicals (tannins, saponins, alkaloids, anthraquinones etc) present in them. Tannins are a polyphenolic compound, reported to produce anthelmintic activity by attaching to the glycoproteins found on the parasitic worm's cuticle thus reducing the amount of glucose available for metabolism or energy supply (Pharmacopoeia of India, 1996). Alkaloids were reported to cause paralysis by acting on the central nervous system as well as permeability of the cell membrane of worms (Roy, 2010; Wang, 2010). Saponins are known to induce and disrupt the selective permeability of the cell membrane in a similar way to the anthelmintic drug. A few synthetic phenolic anthelmintics like niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by their uncoupling oxidative phosphorylation activity (Devraj, 2011)

The methanol extract of *C. albidum* bark has been found to contain certain phytochemicals namely saponins, alkaloids, tannins (Adewoye *et al.*, 2011) after a preliminary phytochemical screening. The LC/DAD profiles of the methanol extract and chromatographic fractions showed the presence of flavonoids, tannins and terpenoids. Fraction A had a flavonoid as the major compound while the other two fractions had lower levels of this compound. Conversely the more active fractions B and C showed higher levels of tannins than fraction A. These tannins are highest in fraction C and may be responsible for its higher activity than that of fraction B. These tannins may therefore be linked to the activity observed.

It could also be that the observed fast paralysis and death in the methanolic extract of C.*albidum* were as a result of a synergistic activity of these phytochemicals present in it. The ballooning or vacuolar degeneration, sloughing off and or loss of epidermal cells of earthworms observed in histological plates of this study might have been as a result of the saponin content present in the extract. It could also be that the saponin content might have disrupted the cell membrane integrity as well as permeability process or pathway of nutrient on the worm's cuticle. The alkaloidal constituents might be responsible for the degenerated muscle fibres and prompt paralysis observed. The tannins might have caused the rapid loss of motility exhibited by these parasitic worms.

In a similar experiment in which *Carica papaya* (pawpaw) was investigated against gastrointestinal nematode *Ancylostoma caninum*in mice where it was concluded that its' efficacy was as a result of its ability to interfere with digestion and removal of the cuticle (Shaziya and Goyal 2012). Similar findings were observed in an experiment conducted with incubation of *H. polygyrus* adult worm in cysteine proteinases (Thompson and Geary 1995, Goodman and Gilman, 2001).

Most antihelminthic have been proven to kill the 'worms' by either depriving (starving) them of food or paralyzing them. Parasitic worms have been observed to lack the ability to store energy hence they are constantly feeding to meet their metabolic needs. A disruption or interference in this process causes energy depletion which leads to death of worm. They have also been found to die when they are paralyzed as they loose their ability to hold their feeding positions within the host's gut (Shaziya and Goyal 2012). These antihelminthic potentials have been observed in the methanolic extract of C.*albidum* and chromatographic fractions C.

It can be concluded that methanol extract of *C. albidum* has the greatest antihelmintic activity compared with other extracts as well as fraction C showing greater activity compared with other fractions. This study has validated the use of *C.albidum* to treat worm infestation in folklore. The plant, *Chrysophyllum albidum* can also be evaluated for probable promising activities during malarial-helminths co-infestation experiments. Further work is on-going to isolate the bioactive compounds responsible for the observed activity.

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