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Evaluation of anthelmintic potential of *Parkia biglobosa* leaves and seeds extracts against infective larvae and adult of *Haemonchus contortus* of goats

J. G. Josiah[1*], I. C. J. Omalu[1], J. Y. Adama[2], I. A. A. Ejima[1] and O. A. Obi[3]

[1]Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.
[2]Department of Animal Production, Federal University of Technology, Minna, Nigeria.
[3]Department of Biological Sciences, University of Agriculture, Markudi, Benue State, Nigeria.

[*]Corresponding author. Email: ganajames@yahoo.com. Tel: 2348036951530.

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ABSTRACT: Among the parasitic diseases that thrive in warm and humid areas, *Haemonchus contortus* is considered to be the most prevalent and devastating species of small ruminant. The present study was carried out to evaluate the activities of leaves and seeds of *Parkia biglobosa* against adult and larvae (L_3) of *H. contortus*. *In vitro* activities were screened by preparing aqueous and methanol extracts of both leaves and seeds of *P. biglobosa* in comparable to controls. Ten worms were exposed to treatment of each plant extract and Albendazole in separate Petri-dishes with 2, 4, 8, 16 and 32 mg/ml of the same volume. The negative control received Phosphate Buffered Saline (PBS). For infective larvae, 20 L_3 were pipetted into 96-flat-bottomed microtitre plate and mixed with the same volume of different concentrations similar to that of adult. The result revealed that, all the adult worms exposed to 32 mg/ml of Albendazole and plant extracts were found dead at 12 hours; whereas none of the worms was found dead in PBS up to 12 hours post exposure. The result of larvae showed that the leaves and seeds extracts of *P. biglobosa* exposed to L_3 of *H. contortus* exhibited less anthelmintic effects in comparable to Albendazole. Albendazole exhibited anthelmintic effects in a dose dependent manner and about 88.5% of L_3 were found dead at 12 hours post-exposure at concentration of 32 mg /ml. But for plant extracts, at 32 mg/ml, less than 40% of L_3 were found dead at 12 hours post exposure. All L_3 larvae survived in PBS up till 12 hours post exposure. It is therefore, concluded that, 32 mg/ml of aqueous and methanol extracts of leaves and seed of *P. biglobosa* have higher adulticidal activity at 12 hours post exposure but lower larvicidal activity against *H. contortus*. However, it is recommended to carry out the *in vivo* study to assess the toxicological effect and recommended doses in goats.

Key words: Adulticidal, Anthelmintic, *Haemonchus contortus*, larvicidal, *Parkia biglobosa*.

INTRODUCTION

There is setback in production and reproductive performance of livestock as a result of helminthosis (Agaie and Onyeyili, 2007; Dawo and Tibo, 2005). Helminthosis of goats and sheep is among the endoparasite infections that are responsible for economic losses through reduced productivity and increased mortality (Perry et al., 2002). Among the parasitic diseases that thrive in warm and humid areas, *Haemonchus contortus* is considered to be the most prevalent and devastating species (Dey et al., 2015). The losses through reduced productivity of small ruminants due to helminths infections is related to reduction of food intake, stunted growth, reduced work capacity, cost of treatment and control of helminthosis (Pedreira et al., 2006; Odoi et al., 2007; Chaudhary et al., 2007).

The Control of helminthosis is usually based on the use of synthetic anthelmintics, whose effectiveness and consistent use has been limited by high levels of anthelmintic resistance and high cost. The inappropriate use of anthelmintic has contributed significantly to the development of resistance against *H. contortus* and other gastrointestinal helminthes (Dey et al., 2015). The first documentation of anthelmintic resistance was to phenothiazine in 1957 followed by thiobendazole in 1964

(Fleming et al., 2006).

The appearance over the last six decades of populations of parasitic worms that have developed resistance to one or more of the available anthelmintic groups has threatened livestock productivity globally (Kaplan, 2004; Waller, 2006). Of particular concern is the discovery of nematodes which are resistant to the three groups of anthelmintics, and cannot therefore be easily controlled by any of the three classes of drugs. This was first detected in South Africa in sheep, and then in Scotland among Angora goat flocks (Coles et al., 1996), but is now known to be more widely distributed (Wrigley et al., 2006). Nematodes in this category include *H. contortus* and *Trichostrongylus* spp.

There are also some indications that human hookworms, notably *Necator americanus* and *Ancylostoma duodenale* are becoming less sensitive to the benzimidazoles and to pyrantel, respectively (De Clercq et al., 1997; Flohr et al., 2007). The survey carried out by Food and Agriculture Organization (FAO) and the Office Internationale des Epizooties (OIE) in 77 out of 151 OIE member countries, revealed that over 50 per cent of countries are affected by parasite resistance (FAO, 2011). This has led to increasing popularity of herbal de-wormers for gastrointestinal nematodes control (Burke et al., 2009).

Presently, focus on medicinal plant is one of the leading researches globally and substantial evidence has been collected to show the immense potentials of these medicinal plants used in various traditional systems (Adamu et al., 2009; Sandoval-Castro et al., 2012). The knowledge of plants, herbs and spices and their respective and collective roles in promoting health is increasing. If the safety and efficacy of these medicinal plants could be ascertained, they could be an alternative and effectively cheaper approach to the control of helminths infections in animals (Soetan and Aiyelaagbe, 2009). One of these medicinal plants is *Parkia biglobosa.*

Parkia biglobosa popularly known as African locust bean, which grow naturally in West Africa, are one of the economic trees in the Northern part of Nigeria (Builders et al., 2012). The efficacy of various preparations of *P. biglobosa* is widely acclaimed for treatment of various diseases among the Hausa communities of Northern Nigeria (Gronhaug et al., 2008; Tijani et al., 2009). The flowers, fruits, seeds, leaves, stem bark and root barks of *P. biglobosa* are all used medically by traditionalist and herbal medicine healers to treat several metabolic and some non-metabolic disorders like haemorrhages, hypertension and dermatosis (Udobi and Onaolapo, 2009; Tokoudagba et al., 2010), diarrhoea, ulcers, pneumonia, burns, coughs and jaundice (Sacande and Clethero, 2007), Antidiarrheal (Agunu et al., 2005), antibacterial (Millogo-Kone et al., 2008) and wound healing (Adetutu et al., 2011). Though, the seeds and leaves of *P. biglobosa* were used *in vitro* to assessed egg hatch assay of nematode with efficacy (soetan et al., 2011; Josiah et al., 2017) but there is dearth of information as well as lack of scientific evidence as regard leaves and seeds of *P. biglobosa* on infective larvae and adult of *H. contortus*. The present study was therefore, carried out to validate the anthelmintic activity of leaves and seeds of *P. biglobosa* in the light of their use in ethnoveterinary medicine.

MATERIALS AND METHODS

Plant materials

The fresh leaves and seeds of *P. biglobosa* used in this research were obtained in the morning from Bokungi village in Edu Local Government Area of Kwara State, North central, Nigeria in the month of March, 2016 (Figure 1). The plant samples were identified and authenticated by a plant taxonomist, Mr Namadi Sanusi in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen number ABU/7064 were prepared and deposited in the herbarium of the Department for reference purpose.

Preparation of plant materials

The collected leaves and seeds of *P. biglobosa* were washed and air dried under the shade at room temperature for four weeks and thereafter pounded into fine powder using mortar and pestle (Soetan et al., 2011). The powders obtained were stored separately in air tight black polythene bags at room temperature which were later use for extraction and phytochemical screening.

Extraction of plant materials

Two solvents (water and methanol) were used for extraction of plant materials. For each extraction, 200 g of powdered plant material was weighed using a sensitive weighing balance with model number SHP1100313194 2011-07 range 0.01 to 500 g. All the extractions were carried out in the Department of Biological Sciences, Federal University of Technology Minna, Nigeria. The aqueous extraction of leaves and seeds were done using the method of Soetan et al. (2011). The weight by weight (w/w) yield of extract was stored in capped bottle at 4^0C.

The methanol extraction of leaves was prepared each by extracting fine powdered leaves (100 g at a time) with 600 ml of methanol for 4 hours using Soxhlet apparatus (Asuzu and Onu, 1994; Builder et al., 2012). The w/w yield of extract was stored in capped bottle at 4^0 C which was later used for the study. The same procedures were done for methanol extraction of seeds of *P. biglobosa*

Determination of percentage yield

The percentage yield of each of the extract was deter-

Figure 1. Bark, flower pod, leaves, seeds of *P. biglobosa* (Field source).

mined using the formular of Ezekwe et al. (2013) as follows:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{Weight of pulverized leaves and seed}} x\ 100$$

Phytochemical analysis of P. biglobosa leaves and seeds

The qualitative phytochemical screenings of the extracts were carried to identify their active constituents using the standard phytochemical methods of Evans 2002, Zohra et al. (2012) and Ezekwe et al. (2013). The phytochemical tests carried out were alkaloids, flavonoids (Sodium hydroxide Test), saponins (Frothing Test), Tannins (Ferrric chloride Test), Terpenoids (Salkowski test), Anthraquinones (Bontrager's Test), glycosides (Benedict's test), cardiac glycoside (Keller-kiliani Test), phlobatannins, sterols and steroids, carbohydrates (Reducing sugars), Starch, proteins and oils.

Collection of adult and infective larvae stage of *H. contortus* for *in vitro* studies

Adult of *H. contortus* were obtained from abomasums purchased from goats slaughtered in Dogarawa slaughtered slab in Zaria, Nigeria. Abomasums were transported to the Helminthology laboratory in the Department of Parasitology and Entomology, Ahmadu Bello University (ABU) Zaria in a cooler with ice block and then washed immediately for the recovering of adult *H. contortus*. The worms were recovered using the method of Hansen and Perry (1994) which were later washed in distilled water and then suspended in phosphate buffered saline (PBS) made by dissolving 0.85 g of sodium chloride (Nacl) and 1 g glucose in 1 litre of distilled water and allowed for 2 hrous to acclimatize (Ombasa et al., 2012). The sample was divided into two portions. First portion was used for adult motility assay while the second portion was used for the processing of infective larvae.

To the second portion, female worms were separated from male worm by their large size and presence of vulva flap. Female worms were then gently crushed to rupture the uteri in order to release their eggs as described by Van Wyk et al. (2004) and Makun et al. (2008). Eggs were cultured at room temperature in damp heat-sterilized bovine faeces for 7 days to provide development using the method of Makun et al. (2008) and Dey et al. (2015). The culture was later baermannized to recover L_3 larvae at the end of the period. The harvested larvae were stored in water at 4°C which were later used for larval motility inhibition assay.

Adult motility inhibition assay (AMIA)

Adult motility assay was conducted on mature live *H contortus* using the method of Iqbal et al. (2006) and Zaman et al. (2011). Ten (10) worms were exposed in triplicate at each of the following treatment in separate Petri-dishes at room temperature (25 to 30^0 C).

1. Leaves and seeds extracts at 2, 4, 8, 16, and 32 mg /ml concentrations.
2. Positive control (Albendazole): 2, 4, 8, 16, and 32 mg/ml.
3. Negative Control (PBS)

The inhibition of motility and/or mortality of the worms were subjected to the above treatments and were used as the criteria for anthelmintic activity. The motility was recorded after 0, 1, 3, 6, 9 and 12 hours intervals. Finally, the treated worms were kept for 30 minutes in the lukewarm fresh PBS to observe the revival of motility. The numbers of live and dead worms were recorded in all the Petri-dishes.

Larval motility inhibition assay (LMIA)

A total of 20 ml of L_3 suspension in water were gotten and 0.1 ml was taken on microscope slide and counted. Approximately 20 L_3 were counted in 0.1 ml. Then, 0.1 ml suspension containing approximately 20 L_3 were pipetted into 96-flat-bottomed microtitre plate and mixed with the same volume of different concentrations (2, 4, 8, 16 and 32 mg/ml) of each plant extract. The positive control wells also received different concentrations of Albendazole (2, 4, 8, 16 and 32 mg/ml) in place of plant extracts while

Table 1. Percentage yield of aqueous and methanol extracts of seed and leaves of *P. biglobosa.*

Extracts	Initial weight of Pulverized (g)	Final weight of the extracts(g)	W/W yield (%)
Aqueous leaves	200	31.72	15.86
Methanol leaves	200	29.42	14.7
Aqueous seeds	200	34.38	17.19
Methanol seeds	200	42.84	21.4

Table 2. Qualitative phytochemical screening of aqueous and methanol extracts of seed and leaves of *P. biglobosa.*

Constituents	Test methods	CAEL	CMEL	CAES	CMES
Cardiac Glycosides	Keller-Kiliani test	++	+	++	-
Flavonoids	NaoH test	-	+++	-	+
Glycosides	Ferric chloride test	-	-	-	-
Oil	Filter paper test	+	-	+	+++
Protein	Millon reagent test,	+	-	+	++
Reducing Sugar	Fehling test	-	+	-	-
Saponins	Frothing test	+	+	+	-
Tannin (Condensed)	Ferric chloride test	-	++	-	-
Tannin (Hydrolysable)	Ferric chloride test	-	++	-	+
Terpenoid	Salkowski test	+	++	+	++
Triterpenoids	Salkowski test	-	+	-	-

Keys: CAEL- Crude Aqueous Extract Leaves, **CMEL**- Crude Methanol Extract Leaves, **CAES**-Crude Aqueous Extract Seed, **CMES**-Crude Methanol Extract Seed, +++ = Abundance, - = Absent, ++ = Moderate, + = Trace.

negative control plates received only PBS and all in triplicate. The motility was recorded after 0, 1, 3, 6, 9 and 12 hours intervals under microscope. The non-motile (dead) L_3 were identified and the percentage calculated (Dey et al., 2015).

Statistical analysis

The data were computed in tables and charts. For adult and larval motility inhibition assay, probit transformation was performed to transform a typical sigmoid dose response curve to linear function (Hubert and Kerboeuf, 1992). The extract concentration required to prevent 50%, i.e., lethal concentration (LC_{50}) of adult and larval from motility were calculated from the linear regression (for y = 0 on the probit scale) using Microsoft Excel Widow 2007. Number of mortality or survival of adult and infective larvae of the parasites in each group was subjected to one-way analysis of variance (ANOVA) followed by Turkey's post hoc test where necessary. Value of $P< 0.05$ was considered significant. GraphPad Instat version 3.05 Window was used to analyze the data.

RESULTS

The percentage w/w yield of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* are shown in Table 1. The highest and lowest yields were methanol seed and methanol leaves respectively. The phytochemical constituents of both aqueous and methanol extracts of leaves and seeds of *P. biglobosa* are shown in Table 2.

Adult motility inhibition assay (AMIA)

The time (hour) taken for motility/mortality and dose dependant response of worms to different extracts were used as criteria to interpret anthelmintic. There was no mortality when worms were exposed to different concentrations of plant extracts for 2 hours including Albendazole (ABZ) and PBS as shown in Table 3. At 3 hours, 100% mortality of worms occurred when exposed to ABZ at 16 mg/ml concentration but plant extracts did not (Table 3). All the worms exposed to 32 mg/ml of ABZ and plant extracts were found dead at 12 hours; whereas none of the worms was found dead or paralyzed in PBS up to 12 hours post exposure (Table 3). The calculated LC_{50}, correlation coefficient and regression equation at 3, 6, 9 and 12 hours are shown in Tables 4, 5, 6 and 7 respectively. The ranking of potency based on LC_{50} and dose dependant effect (R^2) at a particular hour are show in Table 8. At 12 hours, the top three extracts were ABZ,

Table 3. *In-vitro* effect of different extracts of *P. biglobosa* on survival of adult *H. contortus* of WAD goats in comparison with Albendazole.

Treatments mg/ml	Mean number of survived worms at different hours						
	0 hr	1 hr	3 hrs	6 hrs	9 hrs	12 hrs	Fresh PBS for 30 mins
PBS	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}
Albendazole							
2	10.00±0.0^{a}	10.00±0.0^{a}	5.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
4	10.00±0.0^{a}	10.00±0.0^{a}	4.00±0.0^{b}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
8	10.00±0.0^{a}	10.00±0.0^{a}	3.67±0.33^{b}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
16	10.00±0.0^{a}	10.00±0.0^{a}	0.00±0.0^{c}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
32	10.00±0.0^{a}	10.00±0.0^{a}	0.00±0.0^{c}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}
Aqueous extract of leaves of *P biglobosa*							
2	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}
4	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	9.33±0.33^{a}	8.00±0.00^{b}	8.00±0.00^{b}
8	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	7.67±0.33^{b}	4.67±0.33^{c}	4.67±0.33^{c}
16	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	4.00±0.0^{c}	2.67±0.33^{d}	2.67±0.33^{d}
32	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	7.67±0.33^{b}	3.33±0.33^{c}	0.00±0.0^{e}	0.00±0.0^{e}
Methanol extract of leaves of *P biglobosa*							
2	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}
4	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	9.67±0.33^{a}	5.00±0.0^{b}	5.00±0.0^{b}
8	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	8.00±0.0^{b}	1.67±0.33^{c}	1.67±0.33^{c}
16	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	4.67±0.33^{c}	0.00±0.0^{d}	0.00±0.0^{d}
32	10.00±0.0^{a}	10.00±0.0^{a}	7.67±0.33^{b}	7.33±0.33^{b}	1.67±0.33^{d}	0.00±0.0^{d}	0.00±0.0^{d}
Aqueous extract of Seed of *P biglobosa*							
2	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	5.00±0.00^{a}	5.00±0.00^{a}
4	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	4.67±0.33^{a}	4.67±0.33^{a}
8	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	5.33±0.33^{b}	2.67±0.33^{b}	2.67±0.33^{b}
16	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	4.00±0.0^{c}	0.00±0.0^{c}	0.00±0.0^{c}
32	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	5.33±0.33^{b}	1.67±0.33^{d}	0.00±0.0^{c}	0.00±0.0^{c}
Methanol extract of seeds of *P biglobosa*							
2	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	5.33±0.33^{a}	5.33±0.33^{a}
4	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	3.67±0.33^{b}	3.67±0.33^{b}
8	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	4.33±0.33^{b}	0.00±0.0^{c}	0.00±0.0^{c}
16	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	2.33±0.33^{b}	0.00±0.0^{c}	0.00±0.0^{c}	0.00±0.0^{c}
32	10.00±0.0^{a}	10.00±0.0^{a}	10.00±0.0^{a}	0.00±0.0^{c}	0.00±0.0^{c}	0.00±0.0^{c}	0.00±0.0^{c}

Each treatment group had three replicates having 10 worms each. The values with same superscript in column do not differ significantly at P≥0.05.

Crude methanol extract of seed (CMES) and Crude aqueous extract of seeds (CAES), respectively. All the plant extracts (leaves and seeds of *P. biglobosa*) exhibited anthelmintic activity against adult of *H. contortus.* A wide variation however was recorded in the anthelmintic effects among different plant extracts.

Larval motility inhibition assay (LMIA)

The time (hour) taken for motility/mortality and dose dependant response of worms to different extracts were used as criteria to interpret anthelmintic. The dosages of leaves and seeds extracts of *P. biglobosa* exposed to L_3 of *H. contortus* did not exhibit anthelmintic effects in comparable to Albendazole (reference drug). ABZ exhibited anthelmintic effects in a dose dependent manner and about 88.5% of L_3 were found dead at 12 hour post-exposure at 32 mg/ml (Table 9). Though some of the L_3 larvae were found dead at 9 and 12 hours post exposure to plant extract but less than 40% in the concentrations (2,

Table 4. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on adult *H. contortus* motility and or mortality at 3 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	2.69	0.818	Y=2.270x + 4.015
CAEL	-	-	-
CMEL	204.17	0.506	Y= 2.926x - 1.761
CAES	-	-	-
CMES	-	-	-

Table 5. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on adult *H. contortus* motility and /or mortality at 6 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	1.45	0.888	Y= 1.856x +4.697
CAEL	229.09	0.506	Y= 2.839x - 1.709
CMEL	186.21	0.506	Y= 2.986x -1.797
CAES	134.90	0.506	Y= 3.279x - 1.974
CMES	17.78	0.794	Y= 6.788x - 3.501

Table 6. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on adult *H. contortus* motility and /or mortality at 9 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y=7.37
CAEL	16.22	0.805	Y= 4.143x - 0.029
CMEL	15.14	0.865	Y= 4.451x - 0.273
CAES	17.38	0.833	Y= 5.670x - 1.891
CMES	10.96	0.868	Y= 7.309x - 2.609

Table 7. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on adult *H. contortus* motility and or mortality at 12 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y= 7.37
CAEL	10.47	0.876	Y= 5.357x - 0.440
CMEL	7.59	0.786	Y= 5.652x + 0.039
CAES	2.75	0.863	Y= 2.326x + 3.987
CMES	1.82	0.781	Y= 2.292x + 4.404

4, 8, 16 and 32 mg/ml) used. All L_3 survived in PBS up till 12 hours post exposure (Table 9). At 6 hours, it is only ABZ that resulted to 50% mortality at 25.70 mg/ml concentration with $R^2 = 0.852$. The extracts did not cause any 50% mortality of worms (Table 10). The calculated LC_{50}, correlation of regression and regression values for 9 and 12 hours are shown in Tables 11 and 12 respectively. The ranking of potency of ABZ, leaves and seeds extracts based on their LC_{50} and dose dependant effect (R^2) at a particular hour have been listed in Table 13. It is evident from the data that ABZ, leaves and seeds extracts have dose dependent anthelmintic activity despite differences in

Table 8. Ranking of extracts based on LC_{50} values and Regression Correlation on adult *H. contortus* motility and/ or mortality.

Extracts	Ranking of potency based on LC_{50}				Ranking of potency based on dose dependant effect (R^2 - values)			
	3 hrs	6hrs	9hrs	12hrs	3 hrs	6 hrs	9 hrs	12 hrs
ABZ(contr)	01	01	01	01	01	01	01	01
CAEL	-	05	04	05	-	03	05	02
CMEL	02	04	03	04	02	03	03	04
CAES	-	03	05	03	-	03	04	03
CMES	-	02	02	02	-	02	02	05

Table 9. *In-vitro* effect of different extracts of *P. biglobosa* on survival of L_3 of *Haemonchus contortus* of WAD goats in comparison with Albendazole.

Treatments mg/ml	Mean number of survived L_3 at different hours					
	0 hr	1 hr	3 hrs	6 hrs	9 hrs	12 hrs
PBS	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a
Albendazole						
2	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	18.0±0.0^a
4	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.0±0.0^a	20.0±0.0^a	15.0±0.0^b
8	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.0±0.0^b	15.3±0.3^b	9.67±0.3^c
16	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	15.3±0.3^c	9.3±0.3^c	6.3±0.3^d
32	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	10.0±0.0^d	6.3±0.3^d	2.3±0.3^e
Aqueous extract of leaves of *P biglobosa*						
2	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.00±0.0^a
4	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.67±0.3^b
8	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.00±0.0^b
16	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	17.00±0.0^c
32	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	12.33±0.3^b	14.67±0.3^d
Methanol extract of leaves of *P biglobosa*						
2	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.00±0.0^a	20.00±0.0^a
4	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	19.00±0.0^b
8	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.33±0.3^b	18.00±0.3^c
16	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	17.33±0.3^c	16.33±0.0^d
32	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	13.00±0.0^d	12.33±0.3^e
Aqueous extract of seeds of *P biglobosa*						
2	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.00±0.0^a
4	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.67±0.3^b
8	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	17.33±0.3^c
16	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	16.00±0.0^d
32	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	16.00±0.0^d
Methanol extract of seeds of *P biglobosa*						
2	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.0±0.0^a	20.00±0.0^a
4	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	18.33±0.3^b
8	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	15.33±0.3^c
16	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	14.00±0.0^d
32	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	20.00±0.0^a	15.33±0.3^b	12.00±0.0^e

Each treatment group had three replicates having 20 L_3 larvae each. The values with same superscript in column do not differ significantly at P≥0.05.

Table 10. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on Larvae (L_3) *H. contortus* motility and or mortality at 6 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	25.70	0.871	Y= 4.716x - 1.658
CAEL	-	-	-
CMEL	-	-	-
CAES	-	-	-
CMES	-	-	-

Table 11. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on Larvae (L_3) *H. contortus* motility and or mortality at 9 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	19.50	0.852	Y= 5.296x - 1.815
CAEL	150.49	0.506	Y= 3.126x - 1.882
CMEL	31.62	0.855	Y= 4.329x - 1.491
CAES	-	-	-
CMES	229.09	0.506	Y= 2.839x - 1.709

the level of effect.

DISCUSSION

The active principles of many drugs are secondary metabolites present in plants. These secondary metabolites which are phytochemical constituents are very important when investigating anthelmintic of any plant (Ghani, 1990, Builder et al., 2012). In this study, the phytochemical constituents that constitute aqueous and methanol extracts of leaves of *P. biglobosa* are glycosides, cardiac glycosides, flavonoids, oils, reducing sugar, tannin (condensed and hydrolysable), terpenoids and triterpenoids. This result is similar to that of Komolafe et al. (2013) where they obtained the same phytochemical constituents when they used aqueous-methanolic extract of *P. biglobosa* leaves, though alkaloid was absent in this work. Thus the absence may not be a minus for the medicinal efficacies of leaves of *P. biglobosa* but could be the methods of processing and geographical location of this plant that might have led to differences in phytochemical constituents in the two works. Similarly, the aqueous and methanol extracts of seed of *P. biglobosa* indicates the presence of cardiac glycosides, oils, proteins, saponins and terpenoid. The phytochemical constituents of aqueous seed extract are quite similar to the result of Soetan et al. (2011). The only difference is the absent of alkaloid and present of oils, proteins and terpenoid in this work. These results also compared well with those of Abagale et al. (2013) in water and ethanol extracts of fruit husk of *P. biglobosa* where they have cardiac glycosides, oils, proteins, saponins and terpenoid.

Perturbation induced by anthelmintic plants on adult worms survival or their prolificacy that constitute the pathogenic stage could be an important element in parasites struggle. In this study, all treatments base on extracts of leaves and seed of *P. biglobosa* exhibited varying degree of anthelmintic activity. Methanol extract of seeds were found to have higher effects *in vitro* against adult worms when compared to methanol leaves, aqueous leaves and aqueous seed extracts. Methanol extract of seed were found to have 100% mortality of worm when exposed to 8 mg/ml at 12 hours post-exposure. The aqueous and methanol extracts of leaves and aqueous extract of seeds of *P biglobosa* also showed 100% adult mortality at 32 mg/ml concentration but at 12 hours post exposure. The anthelmintic activity in this study is higher than the one found in the study of Marie-Magdeleine et al. (2009) using aqueous extract of seeds of *Cucurbita moschata.* Their result showed 30.4% inhibition of adult *H. contortus* worm motility after 24 hours post exposure to this extract. But, in a more recent study conducted by Dedehou *et al*. (2014) using the extracts of pods fruit of *P. biglobosa* and leaves of *Pterocarpus erinaceus,* 100% of adult worms mobility was inhibited after 36 hours of incubation.

The result of this study also indicated that exposure of adult worms to 32 mg/ml concentration of Albendazole (ABZ), crude aqueous extract leaves (CAEL), crude methanol extract leaves (CMEL), crude aqueous extract seeds (CAES) and crude methanol extract seeds (CMES) of *P. biglobosa* for 12 hours, lead to 100% inhibition of the parasites motility. This result contradicts that of Bogning et al. (2016) who reported 16.67% of inhibition of the parasite

Table 12. $LC_{50,}$ Correlation coefficient (R^2) and regression equation of the effect of different extracts on Larvae (L_3) *H. contortus* motility and or mortality at 12 hours.

Extracts	LC_{50} (mg/ml)	Correlation coefficient (R^2)	Regression equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	8.51	0.995	Y= 2.006x +3.139
CAEL	33.11	0.673	Y= 3.046x +0.37
CMEL	28.18	0.751	Y= 3.338x +0.158
CAES	33.88	0.632	Y= 2.958x + 0.473
CMES	22.91	0.701	Y= 3.432x 0.320

Table 13. Ranking of extracts based on LC_{50} values and Correlation coefficient on Larvae (L_3) *H. contortus* motility and or mortality.

Extracts	Ranking of potency based on LC_{50}			Ranking of potency based on dose dependant effect (R^2 - values)		
	6 hrs	9 hrs	12 hrs	6 hrs	9 hrs	12 hrs
ABZ(Contr)	01	01	01	01	02	01
CAEL	-	03	04	-	03	04
CMEL	-	02	03	-	01	02
CAES	-	-	05	-	-	05
CMES	-	04	02	-	03	03

motility when exposed to highest concentration of the aqueous extract (2400 ug/ml) of *Crassocephalum crepidioides* for 12 hours and 100% inhibition after exposure to 30 hours of incubation. At 6 hours post exposure, the reference drug Albendazole showed 100% mortality at a concentration of 2 mg/ml while the negative control PBS showed no mortality. Additionally, the higher concentrations resulted in early onset of activity and higher number of dead worms when compared to lower concentrations. This suggested that the ABZ and extracts response were time and concentration dependent.

Aqueous and methanol Extracts of leaves and seeds of *P. biglobosa* possessed higher aldulticidal properties with most effective LC_{50} values to be 1.82, 2.75, 7.59 and 10.47 mg/ml for CMES, CAES, CMEL and CAEL respectively at 12 hours post exposure. The study showed that efficacy of extracts increased with increasing concentration of extract and incubation period. Increasing motility inhibition with increasing concentration could be due to the saturation of target receptors. It is likely that at higher concentration, all binding receptors on the worms were occupied thus leading to hyperpolarisation of membranes limiting excitation and impulse transmission causing flaccid paralysis of worm muscles. A similar observation was made by Wasswa and Olila (2006).

In vitro test using the larvae of *H. contortus* is considered to be one of the best means of screening drugs for anthelmintic activity (Asase et al., 2005). They are the infective stage and can be at the origin of the losses of production at the host (Paolini et al., 2003, Brunet et al., 2007). The aqueous and methanol extracts of leaves and seeds of *P. biglobosa* inhibited significantly larval migration of L_3 in comparison to negative control. In this study, in all concentrations (2 to 32 mg/ml) used, there was no mortality or inhibition of motility of larvae when exposed to PBS, ABZ and all the extracts (CAEL CMEL, CAES and CMES) of *P. biglobosa* at 3 hours post exposure. But at 12 hours post exposure, the percentage inhibition of larval migration at concentration of 32 mg/ml were 26.65%, 18.53%, 20% and 40% for CAEL, CMEL, CAES and CMES respectively. The aqueous and methanolic extracts of seeds of *Cucurbita moschata* tested on LMI assay, using the same parasite showed similar results with 21.32% and 33.53% inhibition of larvae respectively (Marie-Magdeleine et al., 2009). However, at 12 hours post exposure, reference drug exhibited 88.5% inhibition of larval migration at 32 mg/ml. This indicates that the extracts showed lesser anthelmintic activity in comparison to reference drug. At 32 mg/ml, less than 40% of L_3 were found dead at 12 hour post exposure when exposed to plant extracts. All L_3 larvae survived in PBS up till 12 hours post exposure.

The LC_{50} determination of larva motility suggested a wide difference in the anthelmintic effect among the different extracts as far as the time and dose dependent effects are concerned. The anthelmintic was observed most at 12 hours post exposure and 50% of L_3 were inhibited at concentration of 8.51, 22.91, 28.18, 33.11 and 33.88 mg/ml for ABZ, CMES, CMEL, CAEL and CAES respectively. As far as ascertained, this is the first scientific evidence of the anthelmintic of CAEL, CMEL, CAES and CMES of *P. biglobosa* against infective larvae.

In general, it is important to note that no work to date on the *in vitro* study of leaves and seeds extracts of *P. biglobosa* on adult and infective larvae of *H. contortus* have been reported. This might be the first scientific evidence of its kind in which leaves and seeds of *P. biglobosa* were used against adult and infective larvae of *H. contortus*. The anthelmintic activity observed in this study was caused by bioactive compounds present in the plant extracts. The larvicidal and adulticidal properties of these extracts may be due to the penetration of active compounds across the cuticle of the parasites on one hand or the absorption of the substance by the parasites through the mouth on the other hand. Enriquez et al. (1993) mentioned that active compounds penetrate the cuticle of nematodes and prevent the absorption of glucose or block the post-synaptic receptors, thus, paralyzing the parasites.

These active compounds can also stimulate the secretion of glutamate and gamma amino-butyric acid (GABA) which may block the transmission of nervous impulses or decoupling the phosphorylation oxidative reactions, which led to energy exhaustion of the parasite thus leading to death (Wabo et al., 2011). Adama et al. (2009) mentioned that active compounds found with food can cross the intestinal lining of larvae and gain access to the circulatory system of the organism. Also, active compounds like tannin may bind to the cuticle of the nematode, destabilize the membrane and increase cell permeability by combining with membrane-associated sterols (Price et al., 1987; Gee and Johnson, 1988) which lead to death. Also, the biological effects of saponins are normally ascribed due to their interaction with the cell membranes, causing changes within the cell membranes, changes in the cell wall permeability and interaction with the collagen proteins from the cuticle of nematodes (Lukhoba et al., 2006; Hernandez-Villegas et al., 2011).

Additionally, these plant extracts (especially methanol leaves and seeds of *P. biglobosa*) contain others major metabolites affecting the migration of adult and L_3 larvae of *H. contortus.* The adult and larval migration might also have been inhibited either by triterpens, or by flavonoids and cardiac glycosides (Ademola et al., 2005; Barrau et al., 2005; Azando et al., 2011). Furthermore, Ayers et al. (2008) showed the contribution of phenols and flavonoïds with anthelmintic activity of *Struthiola argentea*. Thus, the higher flavonoids and saponins present in the extracts of *P. biglobosa* especially in methanol leaves could be actively associated to anthelmintic activity observed. Tannins can also inhibit oxidative phosphorylation, thus decrease metabolism and availability of energy leading to death of the larvae and adult (Athanasiadou et al., 2001).

Conclusion and recommendation

The overall findings of the study showed that the CAEL and CMEL exhibited *in vitro* anthelmintic (100% mortality) against adult *H. contortus* when exposed to 32 mg/ml and 16 mg/ml concentration for 12 hours respectively. For CAES and CMES, 100% efficacy was recorded at 16 mg/ml and 8 mg/ml concentrations when exposed to adult *H. contortus* at 12 hours respectively. This justifies their traditional ethno-veterinary use. In contrast, the *in vitro* anthelmintic activity against infective larvae of *H. contortus* was less efficacious in both the aqueous and methanol extracts. However, the potency of plant extracts was dependence on the time of exposure and concentration of the extracts as well as the solvent used to extract the active ingredients. It is therefore, concluded that, 32 mg/ml of aqueous and methanol extracts of leaves and seed of *P. biglobosa* have higher adulticidal activity at 12 hours post exposure but lower larvicidal activity against *H. contortus*. However, further studies are needed to carry out the *in vivo* study to assess the toxicological effect and recommended doses in goats. Moreover, phytochemical constituents can vary considerably between individual plants due to genetic or environmental differences, developmental stages of plant during harvesting, drying process and storage techniques. Thus, a quality control of the plant materials and extraction itself is strongly recommended for further studies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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