In vitro OVICIDAL A CTIVITY OF *Parkia biglobosa* SEEDS AND LEAVES EXTRACTS AGAINST *Haemonchus contortus*

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ABSTRACT

The frequent use of synthetic anthelmintic over many years has led to the development of drug resistance to parasitic infection nematodes. This study was carried out to evaluate the ovicidal activity of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* used as anthelmintic. The anthelmintic activity of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* was evaluated *in vitro* using egg hatch assay. The extraction of plant materials and phytochemical screening of extracts were done using standard protocols. Fresh eggs of *Haemonchus contortus* were collected from the abomasal contents of goats slaughtered in abattoir. Approximately, 300 freshly collected *H. contortus* eggs in 0.1 ml of distilled water were distributed into 96 flat bottomed micro titre plates and mixed with equal volume of different concentrations 2, 4, 8, 16 and 32mg/ml of each plant extract and positive control (Albendazole). The negative control received PBS with diluents and the eggs solution. The eggs were incubated in this mixture at 27 ^{0}C for 6 days. The experiment was carried out in triplicate for both controls and extracts. The results revealed that, crude aqueous extract of seed, crude methanol extract of seed and crude aqueous extract of leaves produced 100% inhibition of hatchability of *H. contortus* eggs at 2 mg/ml. The crude methanol extract of leaves only produced 100% inhibition of eggs hatchability at 4 mg/ml but at 2mg/ml, 88.9% of eggs were inhibited from hatching. The higher ovicidal activity in this study, may be attributed to the phytochemical constituents present in the extracts working individually or in synergy. From the study, it can be concluded that aqueous and methanol extracts of seeds and leaves of *P. biglobosa* have ovicidal activity against *H. contortus*. It is therefore, recommended that *in vitro* study should be carried out on other developmental stages of *H. contortus* and possibly *in vivo* study and biosafety potentials of this plant be evaluated in goat.

Keywords: Anthelmintic, *Haemonchus contortus, In vitro, Parkia biglobosa*

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INTRODUCTION

Infection by helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (Perry *et al.,* 2002). Among helminth types that infect livestock, *H. contortus* ranks highest in importance globally. They are considered to be the most prevalent and devastating species, thriving most in warm and humid areas. Death rate due

to acute haemonchosis is very high and may go up to 50% in small ruminants (Tariq *et al.,* 2010; Dey *et al.,* 2015).

Most of the present day parasite control programs are based on combination of chemotherapeutic, grazing management, dietary management, biological control, vaccination and ethno-veterinary treatment (FAO, 2002). However, Chemotherapeutic control practices have evolved a number of problems including resistance of helminthes to various groups of anthelmintics, Chemical residues, toxicity problems, increased cost of treatment, non-adaptability of drugs and non-availability of the medicine in remote areas where small ruminants are reared. To this end, the need for the development of alternate methods for the treatment of helminthiasis cannot be overemphasized (Chartier *et al.*, 2001; Iqbal *et al.*, 2003). One of such alternatives is anthelmintics derived from plant origin.

Anthelmintics derived from plants can be a solution to this world wide problem as they form safe and non toxic agents with an altered site of action (Maciel *et al.,* 2006). *Parkia biglobosa*, commonly called African locust bean which belongs to the family of fabaceae is one of the plants used for nutritional and medicinal purposes (Mertz *et al.*, 2001). The potency of various preparation of *P. biglobosa* is widely acclaimed by northern Nigerians, especially among Hausa communities, for the treatment of diseases such as diabetes mellitus, malaria, pain, diarrhoea and hypertention (Tijani *et al.*, 2009). The seed is not usually used as medicine but for its nutritional purposes. Therefore, the research was conducted to investigate the efficacy of seeds and leaves extracts of *P. biglobosa* on *Haemonchus contortus* eggs and provides baseline information on their use as an anthelmintic agent.

MATERIALS AND METHODS

Collection, preparation and extraction of plant extracts

The fresh seeds and leaves of *P. biglobosa* were collected in January, 2016 from Bokungi village in Edu Local Government Area of Kwara State. The samples were authenticated at the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, where a voucher number ABU/7064 was deposited in the herbarium for reference purpose.

The samples were separately air dried after washing in the shade at room temperature for one month and powdered using mortar and pestle. The powder obtained was stored in air tight polythene bags for use in phytochemical analysis (Soetan *et al.,* 2011). Both aqueous and methanol extractions were done in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The method of Soetan *et al.* (2011) was used for aqueous extraction. The extract was concentrated using water bath at 65^{0}C. For methanol extraction, 200g powder of seeds and leaves of *P. biglobosa* was exhaustively extracted with methanol in a Soxhlet's apparatus (Builder *et al.,* 2012). The extract was concentrated in water bath at 65^{0}C. The w/w yield of each aqueous and methanol extract was stored in an airtight container at 4 ^{0}C until use.

Qualitative Phytochemical Screening of seeds and leaves of *P. biglobosa*

The phytochemical screening of the extracts was carried out according to the methods of Evans (2002) and Zohra *et al.* (2012). The screening involves detection of alkaloids, flavonoids, saponins, tannins, terpenoids,

anthraquinones, glycosides, cardiac glycoside/cardenolides, phlobatannins, sterols and steroids, carbohydrates, starch, proteins and oils.

Alkaloid: Crude extract (0.1g) was dissolved in 5ml of 1% hydrochloric acid (aqueous) in a steam bath and filtered. Few drops of Wagner's reagent was added to 1 ml of filtrate. The observed reddish brown precipitate indicates the presence of alkaloids

flavonoids: Few drops of 10% sodium hydroxide was added to 2 ml of extract solution in a test tube. Yellow coloration indicates the presence of flavonoids.

Saponin: Extract (1g) was dissolved in 10 ml of distilled water inside test tube and the mixture shaken together. A honeycomb froth that persisted for 10-15 minutes on warming was taken as preliminary evidence for the presence of saponins.

Tannins: About 1g of extract was dissolved in 5ml of distilled water and then filtered. Thereafter, 3-5 drops of 10% ferric chloride solution was added. A green-black precipitate indicates the presence of condensed tannins while blue or brownish-blue precipitate indicates the presence of hydrolysable tannins.

Terpenoids: Extract (0.2 g) was mixed with 2ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicates positive results for the presence of terpenoids.

Anthraquinones: Extract (5g) was dissolved in 10 ml of benzene and the solution eventually filtered. 10% ammonium solution was then be added to the filtrate and the mixture shaken to observe for the presence of pink, red or violet colour in the ammonia lower phase indicating the presence of free hydroxyl anthraquinone.

Glycosides: Add 5 ml of boiled distilled water to 2ml of the extract solution and stirred. This was filtered and 2 ml of filtrate was hydrolysed with a few drops of Conc HCl and the solution was rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's Qualitative reagent and boiled. Reddish brown precipitate indicates the presence of glycoside

Cardiac glycoside: Two (2 ml) of the extract solution was dissolved in 1 ml of 0.1 M glacial acetic acid containing traces of 1% ferric chloride solution. This was transferred into a dry test tube and 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a layer at the bottom and the interphase carefully observed for purple-brown ring. This colour change indicates the presence of desoxy sugars while a pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides

Phlobatannins: Deposition of red precipitate when solution of extract is boiled with 1% of hydrochloric acid indicates the presence of phlobatannins

Reducing sugars: Add 1 ml of water to 1 ml of extract solution. Add 2 drops of boiling Fehling's solution (A and B) to the mixture in a test tube. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars.

Starch: The aqueous extract 5ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

Proteins: About 5 ml of distilled water was added to 0.1 g of the samples (extracts). The mixture was left to stand for 3 hours and then filtered. 2 ml portion of the filtrate was added to 0.1 ml of Millon's reagent and shake and was kept for observation. A yellow precipitate indicates the presence of proteins

Oils: A quantity, 0.1 g of the samples (Extracts) was pressed between filter

papers and the papers observed. Translucency of the filter paper indicates the presence of oils

Assessment of anthelmintic activity (Egg Hatch Assay)

The Egg Hatch Test (EHT) was performed using the methods of Coles *et al.* (1992). Adult *H. contortus* worms were obtained from the abomasal contents of slaughtered goats purchased from Zaria abattoir in Kaduna State, Nigeria. The female worms were separated and suspended in distilled water and later crushed in mortar and pestle to liberate the eggs. After processing, 0.1 ml suspension containing approximately 300 freshly collected *H. contortus* eggs were distributed into 96- flat- bottomed microtitre plate and mixed with the same volume of different concentration (2, 4, 8, 16 and 32 mg/ ml) of each seeds and leaves extracts of *P. biglobosa*. The positive control wells also received different concentration of Albendazole (24, 8, 16 and, 32 mg /ml) in place of plant extracts while negative control plates received Phosphate Buffer Saline (PBS) as diluent and the egg solution. The eggs were incubated in this mixture at 27^{0}C. After 6 days, a drop of Lugol's iodine solution was added to stop the eggs from hatching. All the eggs, first-stage larvae (L_1) and second-stage larvae (L_2) in each plate were counted. The experiment was carried out in triplicate for both controls and extracts.

RESULTS

The percentage yield of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* are shown in Table 1. The methanol seeds had the highest yield while the methanol leaves had the least yield. The phytochemical screening of the extracts showed the presence of secondary metabolites (Table 2). The 96-well micro titre plates with all extracts including positive control recorded 100% egg inhibition hatch better with inhibition of egg hatch at different concentrations. The only exception is 2 mg/ml of crude methanol extract of leaves (CMEL) and negative control (PBS) that inhibited 88.9% and 16.04 % of eggs hatch respectively (Table 3)

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 seeds	200	34.38	17.19
Methanol seeds	200	42.84	21.40
Aqueous leaves	200	31.72	15.86
Methanol leaves	200	29.42	14.7

the *in vitro* activity of *Peltophorum africanum* Sond (Fabaceae) extract on the egg hatching and larval development of the parasitic nematode (*Trichostrongylus colubriformis*).

From this study, it is evident that, all the aqueous and methanol extracts of seeds and leaves of *P. biglobosa* had higher *in vitro* anthelmintic inhibition of hatching of *H. contortus* eggs. This was confirmed from the negative control (PBS) where 16.4% of *H. contortus* eggs were inhibited from hatching. The higher ovicidal activity may be attributed to the presence of secondary metabolites (tannin, saponins, terpenoid, flavonoid, cardiac glycosides and oils) in the extracts working individually or in synergy. The presence of saponins and tannins in the leaf extracts of *Parkia biglobosa* inhibited the hatching of nematode eggs of ruminant parasites (Soetan *et al.*, 2011). The condensed tannins (CT) extracted from different forages have the ability to inhibit the development of *T. columbriformis* eggs at larva stage 1 (L_1) to larva stage 3 (L_3) (Molan *et al.*, 2002). They suggested that the presence of condensed tannins on forages may have the ability to break the life cycle of sheep nematodes and reduce pasture contamination with infective larva. This may reduce dependence on anthelmintic drugs of controlling helminthes. Saponins are known to destabilize cell membranes hence stop the eggs of *H. contortus* from hatching (Eguale *et al.*, 2007).

The ovicidal effects of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* suggest that the plant extracts have bioactive molecules that could affect the biology of parasitic eggs when sprayed. The results of this study may be significant as the inhibition of egg hatch is an important method of reducing pasture contamination by the animals during grazing helping in the helminthes control.

CONCLUSION

The aqueous and methanol extracts of seeds and leaves of *P. biglobosa* have ovicidal activity against eggs of *H. contortus* and this anthelmintic potential justify their traditional ethno-veterinary use. However, further studies are required *in vitro* on other developmental stages of *H. contortus*. Additionally, *in vivo* anthelmitic effects of *P. biglobosa* plant should be evaluated in goats as well as its biosafety potentials.

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

Constituents	Test methods	CAS	CMS	CAL	CML
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= - Absent, + Present, ++ Very present, +++ much present, CAS-Crude Aqueous Seed, CMS-Crude Methanol Seed, CAL-Crude Aqueous Leaves, CML- Crude Methanol Leaves

Table 3: Comparative inhibitory effects of seeds and leaves extract of *P. biglobosa* on *Haemonchus contortus* eggs

Treatment(Conc-mg/ml)	% inhibition of eggs hatched when exposed to different extracts					
	CAS	CMS	CAL	CML	ABZ	PBS
2	100	100	100	88.9	100	
4	100	100	100	100	100	16.04%
8	100	100	100	100	100	
16	100	100	100	100	100	
32	100	100	100	100	100	

Each data represents percentage eggs inhibited from hatching when exposed to different concentrations of extracts for 6 days.

DISCUSSION

Many researchers have reported the activities of *in vitro* anthelmintic study of plant extracts for the treatment of gastro-intestinal helminths of animals. The results in this study showed that almost all the concentrations (2, 4, 8, 16 and 32mg/ml) of aqueous and methanol extracts of seeds and leaves of *P. biglobosa* exhibited ovicidal activity. Hundred percent (100%) of *H. contortus* eggs were inhibited from hatching when exposed to CAES, CMES and CAEL of *P. biglobosa* at concentration of 2 mg/ml. For CMEL, 100% inhibition occurred at 4 mg/mg but at 2mg/ml, 88.9% of eggs were inhibited from hatching. This showed a dose dependent effect. The control drug Albendazole showed both ovicidal activities at the doses tested and almost all the eggs were found inhibited from hatching. In comparison, the standard drug (ABZ), CAES, CMES and CAEL have the same 100% inhibition of egg hatch at 2, 4, 8, 16 and 32 mg/ml concentration. These observations are quite similar with several investigators. Soetal *et al.* (2011) reported the comparative assessment of *in vitro* anthelmintic effects of the aqueous extracts of seed and leaves of *P. biglobosa* on bovine nematode eggs and their findings showed that the seed extract has more anthelmintic potential than the leaves extract. In this result, the aqueous extracts of seed and leaves have almost the same anthelmintic potential at the same concentration, except the methanol seed extract that has more anthelmintic potential than methanol leaves at 2 mg/ml. Bizimenyera *et al.* (2006) also reported

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