Vol. 22(1), pp.26-38, January 2023
DOI: 10.5897/AJB2022.17528
Article Number: 414CF9870328
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

In vitro anthelmintic activities of stem and root barks extracts of *Parkia biglobosa* on infective larvae and adult of *Haemonchus contortus*

J. G Josiah[1*], J. Y Adama[2,3], Z. Jiya[4], O. M. Abah[5] and C. Imoisi[6]

[1]Department of Biotechnology, Mewar International University (MIU), Masaka, Nasarawa State, Nigeria.
[2]Department of Animal Production, Federal University of Technology, Minna, Niger State, Nigeria.
[3]Africa Centre of Excellence for Mycotoxin and Food Safety (ACEMFS), Federal University of Technology, Minna, Niger State, Nigeria.
[4]Department of Science Laboratory Technology, Federal Polytechnic, Nasarawa, Nasarawa State, Nigeria.
[5]Department of Animal Biology, Federal University of Technology, Minna, Niger State, Nigeria.
[6]Department of Industrial Chemistry, Mewar International University (MIU), Masaka, Nasarawa State, Nigeria.

Received 7 October, 2022; Accepted 22 November, 2022

The treatment of gastrointestinal nematode infections in the 21st century is largely through the use of modern synthetic anthelmintics. However, there is over growing drug resistance to anthelmintics in treatment of gastrointestinal nematode infections in goats and sheep. This present study was carried out to determine the in vitro anthelmintic activitities of Parkia biglobosa using Larval motility inhibition assay (LMIA) and Adult motility inhibition assay (AMIA). The stem and root barks of P. biglobosa were extracted which yielded four different extracts as Crude Aqueous Stem Bark Extract (CASBE), Crude Methanol Stem Bark Extract (CMSBE), Crude Aqueous Root Bark Extract (CARBE) and Crude Methanol Root Bark Extract (CMRBE). The infective larvae and adults of Haemonchus contortus were exposed to different concentrations each (2, 4, 8. 16 and 32 mg/ml) of plant extracts of both stem and root barks of P. biglobosa in comparable to controls (Albendazole (ABZ)-positive control and Phosphate Buffered Saline (PBS)- negative control). The result of LMIA showed that at 12 hours exposure of larvae to 32 mg/ml for four extracts, 55-75% mortality were recorded while the result for AMIA revealed that at 12 hours post exposure of all the plant extracts ranged from 2-32 mg/ml concentrations, 100% mortality of the adult worms were recorded. There was no mortality recorded in negative control (PBS) even up to 12 hours post exposure. From this result, it could be concluded that plant extracts have anthelmintic activities in comparison to ABZ with CMSBE ranked highest among the extracts. However, the potency of plant extracts was dependent on the time of exposure and concentration of the extracts as well as the solvent used.

Key words: Adulticidal, Anthelmintic, Drug resistance, Extracts, in vitro, Larvicidal, Parkia biglobosa

INTRODUCTION

Among the helminth parasites that infect small ruminants, *Haemonchus contortus* is said to be considered the most devastating and prevalent species (Dey et al., 2015). This has led to a major constraint in the production and profitability of small ruminant in Nigeria and African at large (Chiejina, 2001). The devastating and debilitating nature of *H. contortus* is as a result of disease called haemonchosis, it is considered to be the major culprit responsible for hypoproteinemia and anaemia in ruminant animals with heavier worm burdens. However, clinical signs such as weight loss, diarrhoea, anaemia, or sub-mandibular oedema (bottle jaw) may develop (Sissay, 2007).

It has been estimated that a single worm of *H. contortus* sucks about 0.05 ml of blood per day by seepage or ingestion from the lesions (Urquhart et al., 2000). Studies conducted in many countries around the world indicated that among the domesticated animals, goats and sheep suffer mostly from haemonchosis (Nwosu et al., 2007; Tariq et al., 2008). It is important to note that, the infection caused by *H. contortus* ranks highest in global index and capable of causing acute disease and high mortality in all classes of livestock. Death rate due to acute haemonchosis is very high and may go up to 50% in small ruminants (Itty et al., 1997; Perry et al., 2002; Tariq et al., 2010).

Therefore, to minimize the infection caused by *H. contortus* to small ruminants in order to increase their production for protein and economic gain, there is need to develop sustainable control strategies that will reduce or cure the helminthic infections. The management control of nematodes in livestock is basically through systemic synthetic anthelmintics. However, treatments with conventional drugs have their own disadvantages which range from the development of drugs resistance by these parasites, unaffordability of these drugs to low-income farmers as well as accumulation of drug residue in food chain and the environment (Zajac and Gipson, 2000; Vaele, 2002; Schoenian, 2005; Athanasiadou et al., 2008; Sawleha et al., 2010). Thus, alternative methods for controlling helminth infection need to be developed. One of such alternatives is through the knowledge of ethno veterinary medicines which are available for the treatment of internal parasites but are often neglected in favour of the conventional drugs (Hammond et al., 1997).

The knowledge of medicinal plants and their collective roles in promoting health is increasing. One of these medicinal plants is *Parkia biglobosa* which have revealed several phytochemical constituents that have the potential of treating several diseases (Soetan and Aiyelaagbe, 2009). However, to ascertain this claim, further investigation needs to be conducted before proceeding to invivo test (Asase et al., 2005).

The screening of anthelmintic activity is mainly through *in vitro* tests including larval and adult paralysis/death, egg hatch assays and biochemical tests (Bachaya et al., 2009). *In vitro* tests using the infective larvae of *H. contortus* is considered to be one of the best means of screening drugs for anthelmintic activity before proceeding to *in vivo* test (Asase et al., 2005). Therefore, the present study was carried out to investigate the comparative effect of crude methanolic extracts of stem and root bark of *P. biglobosa* on infective larvae and adult stages of *H. contortus*.

MATERIALS AND METHODS

Source of plant materials and authentication

The fresh stem and root barks of *P. biglobosa* were collected in the month of March 2016 in Tsaragi district of Edu Local Government Area of Kwara State, Nigeria (Figure1). The plant samples were identified and authenticated by a plant taxonomist Mr Namadi Sanusi of the Department of Botany, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The plant samples were collectively given a voucher number ABU/7064 which was deposited in the herbarium for reference purposes.

Preparation of plant extracts

The fresh stem and root barks of *P. biglobosa* were separately washed with water and air dried in the shade at room temperature for one month and thereafter crushed with a mortar and pestle or blender into powder form. These were stored in air tight container for later use as described by Soetan et al. (2011) and Meraiyebu et al. (2013).

Methods of aqueous and methanol extraction

The method of Soetan et al. (2011) was used for aqueous extraction. 200 g of the milled stem bark of *P. biglobosa* was weighed using a sensitive weighing balance ranging from 0.01 to 500 g with Model No. SHP1100313194 2011-07 and poured inside a bowl with cover. Then 2 L of distilled water was added and stirred immediately. Stirring was done every 30 min and after 24 h, the supernatant was first filtered with muslin cloth and later through Whatman filter paper No 1. The filtrate was evaporated using the water bath at 65°C for 6 h. The weight by weight (w/w) yield of the aqueous extract was stored in a capped bottle and preserved inside the refrigerator at 4°C. The same procedures were done for the root bark of *P. biglobosa*.

For the methanol extraction, the stem and root barks of *P. biglobosa* were extracted separately using Soxhlet's apparatus. 100 g of each extract was extracted with 600 ml of methanol for 4 h until all the required grams of extracts were exhausted as described by Asuzu and Onu (1994) and Builders et al. (2012). All the filtrate was evaporated using water bath at 65°C and the w/w yield of the extract was stored in an airtight container at 4°C until use.

Yield of percentage determination of aqueous and methanol extracts

The determination of each of percentage yield of aqueous and methanol extract was calculated using the formula of Anokwuru et

*Corresponding author. E-mail: ganajames@yahoo.com.

Figure 1. Sample of *Parkia biglobosa* tree for sourcing of stem bark and root bark.
Source: Author

al. (2011) and Ezekwe et al. (2013) as follows:

$$\% \text{ Yield} = \frac{\text{Weight of each extract}}{\text{Weight of pulverized of each part of } Parkia\ biglobosa} \text{X100}$$

Qualitative phytochemical screening of aqueous and methanol extracts

The phytochemical screening of the extracts was carried out to identify the constituents using standard phytochemical methods. The screening was carried out on each of crude aqueous and methanol extracts of stem and root bark extracts of *P. biglobosa* to determine the possible presence of alkaloids, flavonoids, saponins, tannins, terpenoids, anthraquinones, glycosides, cardiac glycoside/ cardenolides, phlobatannins, sterols and steroids, carbohydrates, starch, proteins, and oils (Sofowora, 1993; Evans, 2002).

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

Adult worms of *H. contortus* were obtained from the abomasums of slaughtered goats purchased in Zaria abattoir Kaduna State, Nigeria. These abomasums were transported on ice block container to Parasitilogy laboratory in the Department of Parasitology and Entomology, Ahmadu Bello University (ABU) Zaria, Kaduna State, Nigeria. The worms were recovered using the method of Hansen and Perry (1994). These worms 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 L distilled water and allowed for 2 h to acclimatize (Kareru et al., 2012). The adult worms processed were divided into two portions for adult motility inhibition assay (AMIA) and larval motility inhibition assay (LMIA). For the latter portion, the female worms were separated from male worms and the female worms were crushed in a mortar and pestle to liberate the eggs. The eggs were recovered from suspension by the method described by Coles et al. (1992). These 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). After 7 days, the culture was baermannized to harvest the L_3 stages and stored in distilled water at 4°C in the laboratory.

Larval motility inhibition assay (LMIA)

For larval motility inhibition assay, a total of 20 ml of L_3 larvae (infective larvae) 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 in triplicate as follows:

(1) For plant extracts: 2, 4, 8, 16 and 32 mg/ml
(2) For albendazole (the positive control wells): 2, 4, 8, 16 and 32 mg/ml.
(3) Negative control plates received only PBS.

The motility was recorded after 0, 1, 3, 6, 9 and 12 h intervals under microscope. The nonmotile (dead) L_3 was identified and the percentage calculated (Dey et al., 2015).

Adult motility inhibition assay (AMIA)

Adult motility assay was conducted on mature live *H. contortus* following the methods of Iqbal et al. (2006), Muhammad et al. (2011) and Zaman et al. (2012). Ten worms were exposed in triplicate at each of the following treatment in separate Petri-dishes at room temperature (25 to 30°C):

(1) Plant extracts: 2, 4, 8, 16, and 32 mg/ml
(2) Albendazole: 2, 4, 8, 16, and 32 mg/ml
(3) Control (PBS)

The inhibition of motility and/or mortality of the worms were subjected to the aforementioned treatments and were used as the criteria for anthelmintic activity. The motility was recorded after 0, 1, 3, 6, 9 and 12 h intervals. Finally, the treated worms were kept for 30 min 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.

Data analysis

The data gotten were presented in tables and charts. The percentage yields of all the extracts were calculated as well as percentages of larva mortality and adult mortality of *H. contortus*. For larval motility inhibition and adult motility inhibition assays, probit transformation was performed to transform a typical sigmoid dose response curve to linear function (Hubert and Kerboeuf, 1992). The linear regression (for y = 0 on the probit scale) using Microsoft Excel Widow 2007 were used to calculate the extract concentration required to prevent 50%, that is, lethal concentration (LC_{50}) of adult and larval from motility.

RESULTS

The percentage yielded for four different extracts are

Table 1. Percentage yield of aqueous and methanol extracts from pulverized form of stem bark and root bark of *Parkia biglobosa.*

Extracts	Initial weight of pulverized (g)	Final weight of the extracts (g)	W/W yield (%)
CASBE	200	31.1	15.6
CMSBE	200	50.29	25.15
CARBE	200	32.47	16.26
CMRBE	200	27.98	13.9

CASBE: Crude aqueous stem bark extract, CMSBE: crude methanol stem bark extract, CARBE: crude aqueous root bark extract, CMRBE: crude methanol root bark extract.
Source: Authors

Table 2. Qualitative phytochemical screening of stem and root bark extracts of *P. biglobosa.*

Constituents	Test methods	CASBE	CMSBE	CARBE	CMRBE
Alkaloids	Mayer's test	+	+++	-	-
Anthraquinones	Bontrager's test	++	+++	++	+
Cardiac Glycosides	Keller-Kiliani test	+	+	+	+
Flavonoids	NaoH test	-	++	-	+
Glycosides	Benedict's test,	+	+++	-	-
Oil	Filter paper test	+	++	-	-
Protein	Millon reagent test	-	-	+	-
Phlobatannins	Hcl test	+++	++	-	+++
Reducing Sugar	Fehling test	+	++	-	+
Saponins	Frothing test	+	+++	++	++
Starch	Iodine test	+	-	-	-
Sterols and Steroids	Conc H_2So_4 test	-	++	-	+
Tannin (Condensed)	Ferric chloride test	+++	+++	+++	++
Tannin (Hydrolysable)	Ferric chloride test	++	++	+	+
Terpenoid	Salkowski test	-	++	-	+
Triterpenoids	Salkowski test	-	-	-	-

- Absent, + present, ++ very present, +++ much present.
Source: Authors

shown in Table 1. The Crude Methanol Stem Bark Extract (CMSBE) yielded the highest percentage while the least percentage was Crude Methanol Root Bark Extract (CMRBE). The qualitative phytochemical constituents of aqueous and methanol extracts of stem and root bark of *P. biglobosa* are shown in Table 2. All the extracts tested were positive with the presence of at least ten phytochemical constituents with various degrees. More phytochemical constituents were present in CMSBE. The anthelmintic activity present in the extracts might be due to the presence of these compounds.

The percentage mortality of larvae (L_3) of *H. contortus* when exposed to ABZ and different concentrations of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* at different hours are shown in Table 3. There was no larvae (L_3) mortality after 1 to 3 h exposure to ABZ at different concentrations (2, 4, 8, 16 and 32 mg/ml) and CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa*, but at 6 h exposure, 50, 50, 43 and 25% mortality of larvae were recorded for ABZ, CASBE, CMSBE and CMRBE at 32 mg/ml. At 12 h exposure, 88% mortality was recorded when L_3 larvae were exposed to 32 mg/ml concentration of ABZ. Similarly, mortality of 55, 87, 63 and 75% were also recorded when L_3 larvae were exposed to 32 mg/ml of CASBE, CMSBE, CARBE and CMRBE, respectively at 12 h. No mortality of the larvae was recorded in PBS up to 12 h post exposure.

The LC_{50} was determined graphically from the regression equation at different hours of exposure using the probit analysis. The values of $LC_{50,}$ coefficient of determination (R^2) and regression equation of the effect of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) mortality are shown in Tables 4, 5 and 6. At 6 h exposure, the value of LC_{50} for ABZ, CMSBE and CMRBE was 25.70, 144.54 and 58.88 mg/ml, respectively. The regression and coefficient of determination (correlation of regression

Table 3. Percentage mortality of larvae (L_3) of *H. contortus* exposed to stem and root bark extracts of *P. biglobosa* in comparison with albendazole.

Treatment (mg/ml)	% Mortality of L_3 at different hours					
	0 h	**1 h**	**3 h**	**6 h**	**9 h**	**12 h**
Phosphate Buffer Saline (PBS)						
	0	**0**	**0**	**0**	**0**	**0**
Albendazole (ABZ)						
2	0	0	0	0	0	10
4	0	0	0	0	0	25
8	0	0	0	10	23	52
16	0	0	0	13	53	68
32	0	0	0	50	68	88
Crude Aqueous Stem Bark Extract (CASBE) of *P. biglobosa*						
2	0	0	0	0	0	3
4	0	0	0	0	0	12
8	0	0	0	10	8	40
16	0	0	0	13	38	50
32	0	0	0	50	50	55
Crude Methanol Stem Bark Extract (CMSBE) of *P. biglobosa*						
2	0	0	0	0	0	8
4	0	0	0	0	10	33
8	0	0	0	0	35	53
16	0	0	0	0	68	73
32	0	0	0	43	70	87
Crude Aqueous Root Bark Extract (CARBE) of *P. biglobosa*						
2	0	0	0	0	0	0
4	0	0	0	0	5	10
8	0	0	0	0	25	30
16	0	0	0	0	42	52
32	0	0	0	0	53	63
Crude Methanol Roots Bark Extract (CMRBE) of *P biglobosa*						
2	0	0	0	0	0	5
4	0	0	0	0	5	10
8	0	0	0	0	17	23
16	0	0	0	5	55	60
32	0	0	0	25	70	75

Each treatment group had three replicates having 20 L_3 larvae each.
Source: Authors

"R^2") were Y= 4.716x - 1.658, R^2 = 0.871; Y= 3.213x 1.934, R^2 = 0.506; and Y= 3.984x - 2.055, R^2 =0.795, respectively while the LC_{50} and R^2 were not recorded for CASBE and CARBE (Table 4). At 9 h, the lowest concentration that resulted to 50% mortality of larvae of *H. contortus* was 14.79 mg/ml of CMSBE and the highest concentration was 23.99 mg/ml of CASBE (Table 6). The CMSBE resulted to 50% mortality of L_3 of with 14.79 mg/ml concentration while the standard drug (ABZ) resulted to 50% mortality at concentration of 19.50 mg/ml. At 12 h exposure to PBS, ABZ and different extracts shown in Table 6. The lowest concentration of extracts that resulted to 50% mortality was CMSBE at 7.94 mg/ml which was even lower than the standard drug (ABZ) which was 8.51 mg/ml for 50% mortality. While the highest concentration was CARBE at 16.98 mg/ml for 50% mortality. The ranking of potency of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* and standard drug (ABZ) based on their LC_{50} and dose dependant effect (R^2) at different hours of exposure are shown in Table 7. It was evident from the result that the effect of CMSBE was prominent and was ranked first

Table 4. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 6 h.

Treatments (mg/ml)	LC_{50} (mg/ml)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	25.70	0.871	Y= 4.716x - 1.658
CASBE	-	-	-
CMSBE	144.54	0.506	Y= 3.213x - 1.934
CARBE	-	-	-
CMRBE	58.88	0.795	Y= 3.984x - 2.055

PBS: Phosphate buffer saline; ABZ: albendazole; CASBE: crude aqueous stem bark extract; CMSBE: crude methanol stem bark extract; CARBE: crude aqueous root bark extract; CMRBE: Crude methanol root bark extract.
Source: Authors

Table 5. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 9 h.

Treatments (mg/ml)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	19.50	0.852	Y= 5.296x - 1.815
CASBE	23.99	0.874	Y= 4.857x - 1.725
CMSBE	14.79	0.781	Y=4.225x + 0.052
CARBE	19.50	0.781	Y = 3.832x + 0.057
CMRBE	16.98	0.849	Y= 4.234x - 0.207

Source: Authors

Table 6. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on larvae (L_3) *H. contortus* motility and/or mortality at 12 h.

Treatments (mg/ml)	LC_{50} (mg/ml)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	8.51	0.995	Y= 2.006x + 3.139
CASBE	18.62	0.903	Y= 1.718x + 2.813
CMSBE	7.94	0.981	Y= 2.062x + 3.144
CARBE	16.98	0.762	Y= 3.961x + 0.142
CMRBE	14.79	0.973	Y= 2.036x + 2.615

Source: Authors

Table 7. Ranking of stem and root bark extracts and standard drug (ABZ) based on LC_{50} values and coefficient of determination on larvae (L_3) *H. contortus* motility and/or mortality.

Treatments	Ranking of potency based on LC_{50}			Ranking of potency based on dose dependent effect (R^2 - values)		
Duration of exposure	**6 h**	**9 h**	**12 h**	**6 h**	**9 h**	**12 h**
ABZ (Control)	01	03	02	01	02	01
CASBE	-	04	05	-	01	04
CMSBE	03	01	01	03	04	02
CARBE	-	03	04	-	04	05
CMRBE	02	02	03	02	03	03

Source: Authors

Table 8. Percentage mortality of adult *H. contortus* exposed to stem and root bark extracts of *P. biglobosa* in comparison with albendazole (ABZ).

Treatment (mg/ml)	% number of dead worms at different hours					
	0 h	1 h	3 h	6 h	9 h	12 h
Phosphate Buffer Saline (PBS)						
	0	0	0	0	0	0
Albendazole (ABZ)						
2	0	0	50	60	100	100
4	0	0	60	80	100	100
8	0	0	63	83	100	100
16	0	0	100	100	100	100
32	0	0	100	100	100	100
Crude Aqueous Stem Bark Extract (CASBE) of *P. biglobosa*						
2	0	0	0	0	3	100
4	0	0	57	67	73	100
8	0	0	80	90	90	100
16	0	0	100	100	100	100
32	0	0	100	100	100	100
Crude Methanol Stem Bark Extract (CMSBE) of *P. biglobosa*						
2	0	0	43	50	100	100
4	0	0	50	80	100	100
8	0	0	77	83	100	100
16	0	0	80	100	100	100
32	0	0	83	100	100	100
Crude Aqueous Root Bark Extract (CARBE) of *P. biglobosa*						
2	0	0	0	0	100	100
4	0	0	40	50	100	100
8	0	0	50	57	100	100
16	0	0	57	93	100	100
32	0	0	87	100	100	100
Crude Methanol Root Bark Extract (CMRBE) of *P. biglobosa*						
2	0	0	0	20	100	100
4	0	0	0	30	100	100
8	0	0	20	53	100	100
16	0	0	40	80	100	100
32	0	0	70	100	100	100

Source: Authors

based on the LC_{50}, followed by CMRBE and lastly ABZ. At 12 h, the top most effective extract/treatment based on LC_{50} was CMSBE, followed by ABZ and CMRBE, CARBE, and CASBE, respectively in decreasing order while based on dose dependant effect were ABZ, CMSBE, CMRBE, CASBE and CARBE, respectively in decreasing order.

Table 8 shows the percentage mortality of adult *H. contortus* exposed to different concentrations of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* in comparison to the standard drug (ABZ) at different hours. There was no mortality when adult worms were exposed to different concentrations of the extracts for 2 h including the positive and negative controls. The onset killing of 50% of the worm at 3 h began with ABZ at 2 mg/ml followed by CMSBE which killed 43% of the adult worms at the same concentration with that of ABZ. But at 4 mg/ml concentration of CMSBE, 50% of adult of *H. contortus* were killed. At 12 h post exposure of all the plant extracts and ABZ ranged from 2 to 32 mg/ml

Table 9. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 3 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	2.69	0.818	Y=2.270x + 4.015
CASBE	10.47	0.629	Y= 4.224x + 0.697
CMSBE	2.24	0.900	Y= 0.942x + 4.670
CARBE	12.30	0.689	Y= 4.195x + 0.428
CMRBE	20.41	0.867	Y= 5.222x - 1.824

Source: Authors

Table 10. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 6 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	1.45	0.888	Y= 1.856x + 4.697
CASBE	7.41	0.749	Y= 5.506x + 0.236
CMSBE	1.86	0.909	Y=2.074x 4.435
CARBE	8.71	0.803	Y= 5.363x - 0.031
CMRBE	5.62	0.926	Y= 2.580x +3.058

Source: Authors

Table 11. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 9 h.

Treatments (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS(-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y=7.37
CASBE	4.17	0.854	Y=3.390x + 2.892
CMSBE	-	1E-1	Y= 7.37
CARBE	"	"	"
CMRBE	"	"	"

Source: Authors

concentration, 100% mortality of the adult worms were recorded while no mortality was recorded in negative control (PBS) even up to 12 h post exposure.

The LC_{50} and correlation of regression (R^2) at 3 h of adult *H. contortus* exposure to CASBE, CMSBE, CARSE and CMRBE as well as positive and negative control are shown in Table 9. All the four extracts including ABZ at varying concentrations resulted to 50% mortality. At 6 h, 50% mortality of the adult worm was recorded for all the plant extracts and the standard drug at various concentrations with the exception of negative control (Table 10). Furthermore, at 9 h post exposure of standard drug and all the extracts, only CASBE at 4.17 mg/ml concentration resulted to 50% mortality of adult *H. contortus*, the remaining extracts and ABZ resulted to 100% mortality of all adult *H. contortus* shown in Table 11. Also, in Table 12, at 12 h post exposure of all the extracts and ABZ to adult *H. contortus*, 100% mortality was recorded. The LC_{50} could not be determined.

The ranking of potency of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* and ABZ based on LC_{50} and dose dependant effect (R^2 values) are shown in Table 13. At 3 h of exposure, CMSBE was ranked the highest for LC_{50} and R^2, followed by ABZ. But at 6 h exposure, LC_{50} was ranked first for ABZ, followed by CMSBE while for R^2, CMRBE was ranked first, followed by CMSBE and ABZ, respectively. It is interesting to know that at 12 h exposure, LC_{50} and R^2 were all ranked first for all plant extracts and ABZ. It was also evident from the data that all the extracts from different parts of the plant have dose dependant anthelmintic activity despite the varied changes.

Table 12. LC_{50}, coefficient of determination (R^2) and regression equation of the effect of stem and root bark extracts of *P. biglobosa* as well as standard drug (ABZ) on adult *H. contortus* motility and/or mortality at 12 h.

Treatment (mg/mL)	LC_{50} (mg/mL)	Coefficient of Determination (R^2)	Regression Equation
PBS (-Ve Control)	-	-	-
ABZ (+Ve Control)	-	1E-1	Y= 7.37
CASBE	-	1E-1	Y= 7.37
CMEBE	-	1E-1	Y= 7.37
CARBE	"	"	"
CMRBE	"	"	"

Source: Authors

Table 13. Ranking of stem bark extracts, root bark extracts and Standard Drug (Albendazole) based on LC_{50} values and coefficient of determination on adult *H. contortus* motility and/or mortality.

Treatment (mg/mL)	Ranking of potency based on LC_{50}				Ranking of potency based on dose dependent effect (R^2 - values)			
Duration	3 h	6 h	9 h	12 h	3 h	6 h	9 h	12 h
ABZ (Control)	02	01	01	01	02	03	01	01
CASBE	03	04	02	01	05	05	02	01
CMSBE	01	02	01	01	01	02	01	01
CARBE	04	05	01	01	04	04	01	01
CMRBE	05	03	01	01	03	01	01	01

Source: Authors

DISCUSSION

Different methods exist for the extraction and separation of plant materials for pharmacological and medicinal uses. In this study, exhaustive extraction of the dried powdered material of stem and root barks of *P. biglobosa* were extracted with water and methanol separately. Among the extraction of aqueous and methanol extracts of *P. biglobosa*, CMSBE gave the highest yield (25.15%) while the CMRBE gave the lowest yield (13.9%). The highest yield reported could possibly be as a result of stem bark of *P. biglobosa* having more phytochemical constituents whose polarity corresponded to that of methanol (Kimani et al., 2013). The percentage yield in this result is higher than that of Salit et al. (2014) who reported 14.5 and 4.0% of yield extracts of seed-husk and stem bark of *P. biglobosa* plant, respectively.

Right from the time immemorial, plants formed part of therapy against parasitic infections of both humans and animals (Priya et al., 2015). Therefore, the basic phytochemicals investigations of extracts of different components of *P. biglobosa* for their major phyto-constituents are important in order to know the secondary metabolites present in this plant.

In this study, the phytochemical constituents present in water and methanol stem bark extracts of *P. biglobosa* were alkaloid, anthraquinones, cardiac glycosides, glycosides, flavonoids, oils, phlobatannins, reducing sugar, saponins, starch, sterols/steroids, tannin (condensed and hydrolysable), and terpenoids. The presence and/or absence of these phytochemical constituents vary in each extract. The results in this study were similar with the results of Ezekwe et al. (2013) in methanol extracts of stem bark of *P. biglobosa*. Millogo Kone et al. (2006) also reported the presence of saponins, glycosides, tannins and other phenolics with trace quantity of alkaloids while Banwo et al. (2004) confirmed the same.

However, the report of Builders et al. (2012) differed slightly from the result of this study due to absence of alkaloids from the methanol stem bark extracts. Thus, the absence may not be a minus for the medicinal efficacies of stem bark 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, in aqueous and methanol extracts of root bark of *P. biglobosa*, the phytochemical constituents present were anthraquinones, cardiac glycosides, flavonoids, phlobatannins, protein, reducing sugar, saponins, sterols/steroids, tannin (condensed and hydrolysable), and terpenoids. These results coincided with the report of Udobi and Onaolapo (2009), who used aqueous and petroleum ether solvents for extraction *P. biglobosa*, although, anthraquinone was absent in their result. It is therefore, important to note that from the four different extracts extracted from different plant parts of *P. biglobosa,* CMSBE contained more secondary metabolites when compared with CASBE, CARBE and CMRBE. *In vitro* tests using the infective larvae of *H. contortus* is considered to be one of the best means of screening drugs for anthelmintic activity (Asase et al., 2005). Several researchers (Ademola et al., 2005; Bizimenyera et al., 2006; Soetan et al., 2011) have reported the activities of *in vitro* anthelmintic study of plant extracts for the treatment of gastro-intestinal helminths of animals. Therefore, perturbation induced by anthelmintic plants on infective larvae and adult worm survival or their prolificacy that constitute the pathogenic stage could be an important element in parasites struggle (Josiah et al., 2018).

The *in vitro* screening of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* showed a significant anthelmintic activity against larvae (L_3) of *H. contortus*. In this study, there was no mortality or inhibition of motility of larvae when exposed to PBS, ABZ and all the aqueous and methanol extracts of *P. biglobosa* at 3 h post exposure. These results contradicted the findings of Dey et al. (2015) who worked on *in vitro* anthelmintic effect of some medicinal plants but not *P. biglobosa* against *H. contortus* and reported varied degree of mortality with different concentration of plant extracts in less than 3 h post exposure.

In this study, the highest efficacy was observed in positive control (ABZ) at 32 mg/ml with 88% mortality in 12 h post exposure. This was followed by CMSBE, CMRBE, CARBE and CASBE with 87, 75, 63 and 55% mortality, respectively at 32 mg/ml in 12 h post exposure. There was no mortality in negative control for up to 12 h post-exposure. It is therefore evident that, the positive control (ABZ) and all the extracts are dose and time dependent.

The LC_{50} determination of larva motility suggested that 50% of L_3 larvae were inhibited at concentration of 25.70, 58.88 and 144.54 mg/ml for ABZ, CMRBE and CMSBE, respectively at 6 h post-exposure. As ascertained, this is the first scientific evidence of the anthelmintic activity of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* against larvae (L_3) of *H. contortus* when exposed below or above 3 h. At 12 h post exposure, 50% mortality was recorded for ABZ, CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* at 8.51, 18.62, 7.94, 16.98 and 14.79, respectively. This suggested a wide difference in the anthelmintic effect among the different extracts as far as the time and dose dependent effects are concerned. Therefore, CMSBE has high antihelmintic efficacy when compared with other extracts and even ABZ.

Larvae (L_3) represent an important stage of the parasitic cycle of *H. contortus*. They are the infective stage and could be a source of losses of production in the host (Paolini et al., 2003; Brunet et al., 2007). The decrease in larval migration induced by plant extracts could be due either to larval mortality or to larval paralysis caused by bioactive compounds present in the *P. biglobosa* especially in CMSBE and CMRBE.

Many researchers such as Molan et al. (2000, 2003), Brunet et al. (2007) and Oloumlade et al. (2011), have shown different extracts from plant rich in tannins and terpenoids are responsible for the inhibition of larval migration of *H. contortus* as well as affected the kinetics of unsheathing of strongyle L_3 and consequently reduced the migration ability of ovine nematode larvae.

Additionally, this plant extracts (especially stem bark of *P. biglobosa*) contained others major metabolites affecting the migration of L_3 larvae of *H. contortus.* The larval migration was also inhibited either by saponins (Lukhoba et al., 2006) and triterpens, or by flavonoids and glycosides (Ademola et al., 2005; Barrau et al., 2005; Azando et al., 2011). Furthermore, Ayers et al. (2008) reported 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 stem bark could be actively associated to anthelmintic activity observed.

The mortality induced by CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* on adult *H. contortus* on this study could be an important element in parasites struggle. The concentration range of 2 to 32 mg/ml of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* was used in comparison with positive control - ABZ (2, 4, 8, 16 and 32 mg/ml) and negative control (PBS) on mature live *H. contortus* of goats. The results of this study indicated that exposure of adult worms to at least 2 mg/ml concentration of ABZ, CASBE CMSBE, CARBE and CMRBE of *P. biglobosa* for 12 h post exposure, lead to 100% mortality or inhibition of adult worms. These results were quite similar with that of Dedehou et al. (2014) who reported that the extracts of pods fruit of *P. biglobosa* and leaves of *P. erinaceus* inhibited 100% adult worm motility after 36 h of incubation. However, the result contradicted that of Bogning et al. (2016) who reported 16.67% of inhibition of the parasite motility when exposed to highest concentration of the aqueous extract (2400 µg/ml) of *Crassocephalum crepidioides* for 12 h and 100% inhibition after exposure to 30 h of incubation.

It is important to note that all the extracts resulted in paralysis and mortality of the tested worms at 12 h post exposure. All the worms exposed to ABZ (a standard anthelmintic drug) were found death at 9 h, whereas none of the worms was dead or paralysed in PBS up to 12 h post exposure. The higher concentrations resulted in early onset of activity and higher number of dead worms compared with lower concentrations. This suggested that the extracts response were time and concentration dependent. In this study, CMSBE resulted to onset killing of adult worm when compared with ABZ. This is evidence from the 3 h post exposure where CMSBE was ranked first on potency based on LC_{50}. The tannins contained in plants have been reported to possess anthelmintic (Paolini et al., 2003, 2005; Ademola et al., 2004, 2005) activities. It is postulated that condense tannin may impair vital processes such as feeding and reproduction of the parasite or may bind and disrupt the integrity of the parasites' cuticle (Niezen et al., 1995). In general, it is important to note that the literature is scarce on the *in vitro* study of stem and root barks extracts of *P. biglobosa* on infective larvae and adult of *H. contortus.*

In vitro evaluation for anthelmintic activity of CASBE, CMSBE, CARBE and CMRBE of *P. biglobosa* showed that all extracts exhibited anthelmintic activity against *H. contortus* as evident from larva motility inhibition assay and adult motility assay of the worms. A wide difference, however, was recorded in the anthelmintic effects among different extracts as far as the intensity, time and dose dependent effects were concerned. The larvicidal and adulticidal properties of these extracts may be due to active compounds present in the extracts that penetrate across the cuticle of the parasites on one hand or the absorption of the active compounds by the parasites through the mouth on the other hand. Active compounds could penetrate through the cuticle of nematodes and prevent the absorption of glucose or block the post-synaptic receptors, thus, paralyzing the parasites as mentioned by Enriquez et al. (1993).

Conclusion

The overall findings of the study showed that CASBE, CMSBE, CMRBE and CARBE exhibited *in vitro* anthelmintic of 55, 87, 63 and 75% mortality, respectively against infective larvae of *H. contortus* when exposed to 32 mg/ml concentration for 12 h while 100% mortality was recorded against adult *H. contortus* when exposed to 32 mg/ml concentration of CASBE, CMSBE, CMRBE and CARBE for 6 h with CMSBE ranked the highest in LC_{50} and R^2. The *in vitro* anthelmintic activity against infective larvae of *H. contortus* was less efficacious in both the aqueous and methanol extracts when compared with adult *H. contortus*. However, the potency of plant extracts was dependent 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 stem and root barks of *P. biglobosa* have higher adulticidal activity at 6 h post exposure but lower larvicidal activity against *H. contortus* and this justifies their traditional ethno-veterinary use. However, further studies are needed to carry out the *in vivo* study to assess the toxicological effect on animal model.

ACKNOWLEDGEMENTS

The authors appreciate the students of Etsu Aliyu Senior Secondary School Tsaragi for assistance in sourcing and pounding of stem and root barks of *Parkia biglobosa* plant. They also thank the laboratory technical staff of the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ademola IO, Fagbemi BO, Idowu SO (2004). Evaluation of anthelmintic activity of Khaya senegalensis extract against gastrointestinal nematodes of sheep: *in vitro* and *vivo* studies. Veterinary Parasitol. 122:151-164.

Ademola IO, Fegbemi BO, Idowu SO (2005). Anthelmintic activity of Extracts of Spondias mombin against gastrointestinal nematodes of sheep: Studies in vitro and vivo. Tropical Animal Health Production 37:223-235.

Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P (2011). Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants, Nature and Science 9(7):53-61.

Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MSJ (2005) Ethnobotanical study of some Ghanian anti-malarial plants. Journal of Ethnopharmacology 99(2):273-279.

Asuzu IU, Onu OU (1994). Anthelmintic activity of the ethanolic extract of Piliostigma thonningii bark in Ascaridia galli infected chickens. Fitoterapia 65(4):291-297.

Athanasiadou S, Houdijk J. Kyriazakis I (2008). Exploiting synergisms and interactions in the nutritional approaches to parasite control in sheep production systems. Small Ruminant Research 76(1-2):2-11.

Ayers S, Zink DL, Mohn K, Powell JS, Brown CM, Murphy T, Singh SB (2008). Flavones from *Struthiola argentea* with anthelmintic activity in vitro. Phytochemical Journal 69:541-545.

Azando EVB, Hounzangbe-Adote MS, Olounlade PA, Brunet S, Fabre N, Valentin A, Hoste H (2011). Involvement of tannins and flavonoids in the in vitro effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloïdes* extracts on the exsheathment of third-stage infect tive larvae of gastrointestinal nematodes. Veterinary parasitology 180(3-4):292-297.

Bachaya HA, Iqbal Z, Khan MN, Sindhu Z, Jabbar A (2009). Anthelmintic activity of ziziphus nummularia (bark) and *Acacia nilotica* (fruit) against *Trichostronglyloid nematodes* of sheep. Journal Ethnopharmacol 123(2):325-329.

Banwo GO, Abdullahi I, Duguryil M (2004). The antimicrobial activity of the stem bark of Parkia clappertoniana keay family Leguminosae against selected microorganisms. Nigerian Journal of Pharmarceutical Research 3(1):16-22.

Barrau E, Fabre N, Fouraste I, Hoste H (2005) Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia* Scop.) on the in vitro larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Journal of Parasitology 131(4):531-538.

Bizimenyera ES, Githiori JB, Eloff JN, Swan GE (2006). *In vitro* activity of *Peltophorum africanum* Sond (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. Veterinary Parasitology 142(3-4):336-343.

Bogning ZC, Olounlade PA, Alowanou GG, Nguemfo EL, Dongmo AB, Azebaze AGB, Hounzangbe-Adote S (2016). *In vitro* anthelmintic activity of aqueous extract of *Crassocephalum crepidioides* (Benth.) S. Moore on *Haemonchus contortu*. Journal of Experimental and Integrative Medicine 6(1):31-37.

Brunet S, Aufrere J, Elbabili F, Fouraste I, Hoste H (2007). The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both *in vitro and in vivo*. Parasitology 134(9):1253-1262.

Builders MI, Isichie CO, Aguiyi JC (2012). Toxicity Studies of the Extracts of Stem Bark in Rats. British Journal of Pharmaceutical Research 2(1):1-16.

Chiejina SN (2001). The epidemiology of helminthes infection of domesticated animals in the tropics with emphasis on fascioliasis and parasitic gastroenteritis, In: Chowdhury, N., Tada, I. (Ed): Perspectives on Helmintology. Science Publishers Inc., Enfield pp. 41-87.

Coles GC, Bauer FHM, Borgsteede S, Greerts S, Klei TR, Taylor MA, Waller PJ (1992). World association for the advancement of veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 44(1-2):35-44

Dedehou VFGN, Olounlade PA, Adenile AD, Azando EVB, Alowanou GG, Daga FD, Hounzangbe-Adote MS (2014). Effets in vitro des feuilles de Pterocarpus erinaceus et des cosses de fruits de *Parkia biglobosa* sur deux stades du cycle de développement de *Haemonchus contortus* nématode parasite gastro-intestinal de petits ruminants. Journal of Animal and Plant Science 22(1):3368-3378.

Dey AR, Akther S, Hossain S, Dey TR, Begum N (2015). *In Vitro* Anthelmintic Effect of some Medicinal Plants against *Haemonchus Contortus*. Journal of animal science advances 5(1):1162-1170.

Enriquez FJ, Scarpino V, Cypen RH, Wasson DL (1993). *In vitro* and *in vivo* egg production by Nematospiroides dubuls during primary and challenge infection in resistant and susceptible strain of mice. Journal of Parasitology 74(2):262-266.

Evans WC (ed) (2002). Trease and Evans' Pharmacology 15th Edition, W.B. Saunders, New York pp. 221-393.

Ezekwe CI, Anaya C, Okechukwu PCU (2013). Effects of Methanol Extract of *Parkia biglobosa* Stem Bark on the Liver and Kidney Functions of Albino Rats. Global Jornal of Biotechnology and Biochemistry 8(2):40-50.

Hammond JA, Feelding D, Bishop SC (1997). Prospects for plant antihelmintics in tropical veterinary medicine. Veterinary Research Communication 21:213-228.

Hansen J, Perry B (1994). The Epidemiology, Diagnosis and control of Helminth parasites of ruminants. International Livestock Center for Africa, Addis Ababa Ethiopis pp. 90-100.

Hubert J, Kerbouef D (1992). A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. Veterinary Record 130(20):442-446.

Iqbal Z, Lateef M, Jabbar A, Ghayur MN, Gilani AH (2006). In vitro and in vivo anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. Phytotherapeutic Resource 20:46-48.

Itty PJ, Zinsstag P, Ankers M, Njie E, Pfister K (1997). Returns from Strategic antihelimintic treatments in village sheep in the Gambia. Preventive Veterinary Medicine 32(3-4):299-310.

Josiah JG, Omalu ICJ, Adam JY, Ejima IAA, Obi OA (2018). Evaluation of anthelmintic potential of *Parkia biglobosa* leaves and seeds extracts against infective larvae and adult of *Haemonchus contortus* of goats. Journal of Animal Science and Veterinary Medicine 3:6-17.

Kareru PG, Ombasa O, Rukunga G, Mbaria J, Keriko JM, Njonge FK and Owuor, BO. (2012). in-vitro antihelmintic effects of two kenyan plant extracts against *heamonchus contortus* adult worms, International Journal of Pharmacological Research 2(3).

Kimani D, Kareru PG, Njonge FK, Kutima HL, Nyagah GC, Rechab SO, Wamburu RW, Karanja JM (2013) Control of gastro-intestinal nematodes in ruminants using plant extracts. Scientific conference proceeding pp. 193-198.

Lukhoba CW, Simmonds SJ, Paton AJ (2006). Plectranthus: a review of ethnobotanical uses. Journal of Ethnopharmacology 103:1-24.

Makun HJ, Ajanusi OJ, Lakpini CAM, Ehoche OW, Rekwot PI (2008). Response of Red Sokoto and Sahelian Goats to Trickle *Haemonchus contortus* Infection. Journal of Biological Sciences 8:753-759.

Meraiyebu A, Olaniyan O, Abutu S, Dare J, Atsukwei D (2013). Hepatoprotective Effect of *Parkia Biglobosa* Stem Bark Methanolic Extract on Paracetamol Induced Liver Damage in Wistar Rats. American Journal of Biomedical and Life Sciences 1(4):75-78.

Millogo-Kone H, Guissou IP, Nacoulma O, Traore AS (2006). Study of the antibacterial activity of the stem bark and leaf extract of *Parkia biglobosa* (Jacq) Benth on Staphylococcus aureus. African Journal of Traditional Complentary and Alternative Medicine 3(2)74-78.

Molan AL, Alexander RA, Brookes IM, McNabb WC (2000). Effects of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes *in vitro*. New Zealand Society of Animal Production 60:21-25.

Molan AL, Meagher LP, Spencer PA, Sivakumaran S (2003). Effect of flavan- 3-ols on *in vitro* egg hatching, larval development and viability of infective larvae of Trichostrongylus colubriformis. International Journal of Parasitology 33(14):1691-1698.

Muhammad AZ, Zafar I, Muhammad NK, Ghulam M (2011). Anthelmintic Activity of a Herbal Formulation Against Gastrointestinal Nematodes of Sheep. Pakistan Veterinary Journal pp. 2074-7764.

Niezen JH, Waghorn TS, Charleston WA, Waghorn GC (1995). Growth and gastrointestinal parasitism in lamb grazing one of seven herbages and dosed with larvae for six weeks. Journal of Agricultural Science 125(2):281-289.

Nwosu CO, Madu PP, Richards WS (2007). Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. Veterinary Parasitology 144(1-2):118-124.

Paolini V, Bergeaud JP, Grisez C, Prevot F, Dorchies P, Hoste H (2003). Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Veterinary Parasitology 113(3-4):253-261.

Paolini V, De La Farge F, Prevot F, Dorchies PH, Hoste H (2005). Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. Veterinary Parasitology 127(3-4):277-283.

Perry BD, Randolph TF, McDermott JJ, Sones KR and Thornton PK (2002). Investing in Animal Health Research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya pp. 148.

Priya MN, Darsana U, Sreedevi R, Deepa CK, Sujith S (2015). *In vitro*-ovicidal activity of Allophyllus cobbe leaf extracts against *Haemonchus contortus*. Internatinal Journal of Applied and Pure Science and Agriculture 1(3):24-28.

Salit BS, Emmanuel UO, Idongesit JM, Pau lNO, Labumi L (2014). Parka Biglolosa Plants Parts: Phytochemical, Antimicrobial, Toxicity and Antioxidant Characteristics. Journal of Natural Sciences Research 4(2):130-133.

Sawleha Q, Dixit AK and Pooja D (2010) Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants. Veterinary World 3(11):515-518.

Schoenian S (2005). Maryland small ruminant page internal parasites of sheep and goats. www.sheepandgoats.com/articles/sheepgoatparasites. Retrieved on 12/09/2008.

Sissay MM (2007). Helminth Parasites of Sheep and Goats in Eastern Ethiopia. Doctoral thesis Swedish University of Agricultural Sciences Uppsala.

Soetan, KO and Aiyelaagbe OO (2009). The Need for Bioactivity-Safety Evaluation and Conservation of Medicinal Plants- A Review. Journal of Medical Plants Research 3(5):324-328. Available online at http://www.academicjournals.org/JMPR

Soetan KO, Lasisi OT and Agboluaje, AK (2011). Comparative assessment of in-vitro anthelmintic effects of the aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs. Journal of Cell and Animal Biology 5(6):109-112.

Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria pp. 191-289.

Tariq KA, Chishti MZ, Ahmad F, Shawl AS (2008). Epidemiology of gastro-intestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley Veterinary Parasitology 158(1-2):138-143.

Tariq KA, Chishti MZ, Ahmad F (2010). Gastro-intestinal nematode infections in goats relative to season, host, sex and age from the Kashmir valley. Indian journal of helminthology 84:93-97.

Udobi CE, Onaolapo JA (2009). Phytochemical analysis and antibacterial evaluation of leaf, stem bark and root of the African locust bean (*Parkia biglobosa*). Journal of Medical Plants Resources 3(5):338-344.

Urquhart GM, Armour J, Dunca JL, Dunn AM, Jennings FW (2000). Veterinary Parasitology 2nd Edition. Blackwell Science Limited London.

Vaele PI (2002). Resistance to macrocyclic lactones in nematodes of goats. Australian Veterinary Journal 80(5):303-304.
Zajac AM, Gipson TA (2000). Multiple anthelmintic resistance in a goat herd. Veterinary Parasitology 87:167-172.
Zaman MA, Iqbal Z, Khan MN, Muhammad G (2012). Anthelmintic activity of a herbal formulation against gastrointestinal nematodes of sheep. Pakistan Veterinary Journal 32(1):117-121.