

# ANTHELMINTIC ACTIVITIES OF STEM BARK OF PARKIA BIGLOBOSA ON WEST AFRICAN DWARF GOATS INFECTED WITH HAEMONCHUS CONTORTUS

DJames Gana Josiah<sup>1\*</sup>, D Okechukwu Anthony Obi<sup>2</sup>,

D John Yisa Adama<sup>3</sup>, D Innocent Chukwuemeka James Omalu<sup>4</sup>

 <sup>1</sup>Ahman Pategi University, School of Pre-Degree Studies, Pategi, Nigeria
 <sup>2</sup> University of Agriculture, College of Sciences, Department of Zoology, Markudi, Nigeria
 <sup>3</sup> Federal University of Technology, School of Agricuture and Agricutural Technology, Department of Animal Production, Minna, Nigeria
 <sup>4</sup> Federal University of Technology, School of Life Sciences, Department of Animal Biology, Minna, Nigeria

> \*Corresponding Author: E-mail: ganajames@yahoo.com

(Received 07<sup>th</sup> March 2021; accepted 08<sup>th</sup> October 2021)

ABSTRACT. The anthelmintic effects of stem bark extracts of Parkia biglobosa was evaluated on West African Dwarf (WAD) goats. The stem bark of the plant was obtained from Kwara state of Nigeria. The extraction was done and yielded Crude Methanol Stem Bark Extracts (CMSBE), Ethyl acetate (EA) fraction and Aqueous (AQ) fraction. The anthelmintic potentials were studied in vivo in 18 WAD goats in six completely randomised groups, A, B, C, D, E and F with three animals per group. Group A was treated with 5mL/kg of distilled water as negative control, group B with 6.25mg/kg of Albendazole (ABZ)(positive control), groups C and D with 1000mg/kg and 2000mg/kg of CMSBE respectively and groups E and F with 1000mg/kg of EA fraction and AQ fraction each respectively. Faecal samples were collected for two weeks after treatment to evaluate faecal egg counts. After 16th day, one animal was euthanized from each group to determined percentage deparasitization. The result from this study revealed that, the phytochemical constituents present in CMSBE were alkaloid, anthraquinones, cardiac glycosides, glycosides, flavonoids, oils, phlobatannins, reducing sugar, saponins, sterols/steroids, tannin (condensed and hydrolysable) and terpenoids. In vivo result showed significant (P<0.05) reduction in egg per gram (EPG) faeces in group B, C, D and E when compared to group A, all at 16th day post-treatment. Group F showed less efficacy in EPG reduction. The deparasitisation obtained in groups D and E were higher than group B, though without statistical significant difference. The study has shown that, CMSBE and EA fraction of P. biglobosa exhibited in vivo anthelmintic activities at 2000mg/kg and 1000mg/kg respectively that are sufficiently comparable to ABZ, hence, have potentials as a novel anthelmintic ethnobotanic preparation for control of H. contortus in WAD goats. It is therefore recommended to carry out a further research on larger population size of goats.

Keywords: Egg counts, extract, faecal, goats, phytochemicals

#### **INTRODUCTION**

Infections caused by gastrointestinal parasites pose the greatest challenge to goat health and production all over the world especially in tropical and sub-tropical countries [1]. They cause reduced feed intake, weight loss, reduced immunity, impaired fertility, damage gastric function and high mortality rate which lead to enormous economic losses [2]. In Nigeria, it was estimated that about 40-60% of the lambs die due to gastrointestinal (GI) nematode infections [3]. Consequently, there is need to treat and control infections caused by *Haemonchus*. *contortus* and other nematodes that coexist with them in small ruminants particularly in goats in the tropics.

The treatment and control of these infections in livestock is mainly by chemical anthelmintic which have the disadvantages of being costly for low income and poor farmers. The risk of environmental pollution as a result of residue in the food chain and environment as well as the development of resistance in all major parasites species are also problems [4,5,6]. Thus, alternative methods for control of gastrointestinal nematodes need to be developed. One of such alternative is through the knowledge of ethno veterinary medicine.

The use of sustainable, integrated parasite control systems, using scientifically proven non-chemical methods and limited use of drugs is being considered to ensure animal health and food safety [7]. The research on medicinal plants that contain bioactive compounds to control helminths either as phytotherapeutic or nutraceutical options is one of the leading areas of research globally [8-9]. A comprehensive natural alternative anthelmintic management program that includes the use of plants as a cheaper and sustainable alternative to synthetic drugs would result in beneficial health and economic impacts on the goat industry. Studies show that plant species can effectively reduce the degree of parasite infestation in livestock and are promising alternatives to conventional anthelmintics [10].

Parkia biglobosa commonly called the African locust bean is a leguminous tree which belongs to family fabaceae tree is native to Nigeria and other West African countries. The different components of P. biglobosa are used by traditionalist and herbal medicine healers to treat several metabolic and some non metabolic disorders like haemorrhages, hypertension and dermatosis [11-12]. Recent survey on the ethno-pharmacology carried out in the Northern parts of Nigeria revealed that the stem bark extract was among the commonly used plants used for the treatment of diabetes mellitus by traditional healers [13]. The efficacy of various preparation of *P. biglobosa* is also widely acclaimed by the Hausa communities of Northern Nigeria for the treatment of diseases as malaria, diabetesmellitus and pain [14-15]. If the safety and efficacy of this plant P. biglobosa could be ascertained, they could be an alternative and effectively cheaper approach anthelmintic for the treatment and control of helminth infections in livestock. Therefore, this plant was selected because of their medicinal properties and their continual use in traditional medicine in the treatment of several diseases. Thus, the aims of the present study are to determine the anthelmintic activities of methanol stem bark extract on the West African Dwarf (WAD) goats experimentally infected with H. contortus.

# MATERIALS AND METHODS

# Plant Collection and Authentication

The fresh stem bark of *P. biglobosa* was collected in the month of March, 2016 in Bokungi village in Edu Local Government Area of Kwara State, Nigeria (Fig.1). The plant collected was identified and authenticated in Herbarium in the Department of Botany, Ahmadu Bello University Zaria, Nigeria. The Voucher number ABU/7064 was assigned to the sample which was deposited in the herbarium in the same Department for future references.



Fig. 1. Sourcing of stem bark of P. biglobosa (Field source)

# Plant Preparation and Extraction

The fresh stem bark of *P biglobosa* was washed with water and air dried in the shade at room temperature for one month and thereafter crushed with a mortar and pestle into fine powder. These were stored in air tight container for later use [16]. The powder form (100 g at a time) was extracted with 600 ml of methanol in a Soxhlet's apparatus for 4 hours [17]. All the filtrate was evaporated using water bath at 65°C. The weight by weight (w/w) yield of the extract was stored in a capped bottle and preserved inside the refrigerator at 4°C.

# Extract Partitioning and Determination of Maximum convenient dose (MCD), maximum Convenient Concentrations (MCCs) and Maximum Convenient Volume (MCV)

Crude methanol extract of stem bark (CMESB) of *P. biglobosa* was partitioned [18]. Three solvents used in partitioning were n- Hexane, Ethyl acetate (EA) and water (AQ). In each partitioning step, the mixtures were vigorously shaken to re-suspend the particles. Impurities were pooled together in a separate beaker and discarded. The different portions collected in separate conical flasks were concentrated to residue over the water bath at 65°C and weighed to determined percentage yield. The different fractions were packed in clean air-tight glass bottles and stored in refrigerator at 4°C until used. The maximum convenient doses (MCD) were calculated, due to lack of information on the actual doses of the plant extracts. The maximum convenient concentrations of stem bark of *P. biglobosa* extracts were prepared [19-20] using the methods of Lorke (1983) and Ibrahim (1984). The maximum convenient volume (MCV) that could be administered to the goat by the oral route (gavage) is 5ml/kg [19-21]. The MCDs (g/kg) was calculated by multiplying the MCCs (g/ml) by the MCV (ml/kg) [22].

### Qualitative Screening of Phytochemical Constituents in Plant Extracts

The CMESB and its various fractions were screened to determined the possible presence of alkaloids, flavonoids, saponins, tannins, terpenoids, anthraquinones, glycosides, cardiac glycoside/cardenolides, phlobatannins, sterols and steroids, carbohydrates, starch, proteins and oils. The screening were carried out using the standard phytochemical methods [23-24].

### Recovery of Infective Larvae (L<sub>3</sub>) of H. contortus for Goats Infection

Infective larvae of *H. contortus* were obtained from abomasums purchased from goats slaughtered in Minna Abattoir. Abomasums were then transported to the Biology Laboratory I in the Department of Animal Biology, Federal University of Technology, Minna, in a cooler with ice block and then washed immediately. 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 for culturing. Eggs were cultured at room temperature in damp heat-sterilized bovine faeces for 7 days to provide development. Cultures were baermannized to recover larvae at the end of the period. The harvested larvae were stored in distilled water at 4°C. Thereafter, goats were inoculated accordingly [25].

## Sourcing and Experimental Infection of WAD Goats with L<sub>3</sub> of H. contortus

Eighteen (18) apparently healthy WAD goats with an average weight of 10 kg of both sexes (males and females) which have homogeneous characteristics were purchased from small scale farmers in Niger state. Infections of these goats were confirmed in Biology Laboratory I in the Department of Animal Biology, Federal University of Technology, Minna in collaboration with Niger State Veterinary Hospital Minna [26-27]. After confirmation, any animal with gastrointestinal infection and ectoparasites were dewormed. These animals were then immunized with pestes des petit (PPR) vaccine purchased from the Nigerian Veterinary Research Institute Vom, Plateau State. The animals were maintained in concrete-floored pens constructed in Biological Garden of Centre for Preliminary and Extral-mural Studies, FUT, Minna. The animals were fed with standard diet (fed with cured, cut and carry forage supplemented with maize offal, Groundnut hay, yam peeled, beans husk, salt lick) and water ad libitum. The study was conducted in accordance with the ethical rules on animal experimentation as approved by the Ethical Committee of FUT, Minna. The animals were then acclimatized for two weeks before the commencement of the study [17, 28]. Each animal was inoculated with a 5 ml aliquot of the L<sub>3</sub> of H. contortus parasites solution estimating a dose of 2500 larvae per animal using syringe [29-30]. The inoculums were administrated orally and care was taken to ensure oral administration was as far back in the goat's mouth as possible to reduce expectoration of the solution [31]. Twenty one days after experimental infection, animals were randomly allotted into six treatment groups (A, B, C, D, E and F) of three animals each. The grouping was done using complete randomized design (CRD), taking into consideration their live weight [17]. The six groups were assigned to different treatment groups as follow: group A were administered with 5 mL/kg of distilled water, group B were administered with 6.25 mg/kg of Albendazoles, group C and D were administered with 1000mg/kg and 2000mg/kg of CMSBE of *P. biglobosa*, respectively, group E were administered with 1000mg/kg of EA fraction of CMSBE and 1000mg/kg of AQ fraction were administered to group F.

# Faecal Egg Counts (FEC)

Faecal samples (2g) were collected directly from the rectum of each goat on days 0, 4, 8, 12 and 16. The modified McMaster technique was used for egg count [26-27]. Egg per gramme (EPG) was calculated by multiplying faecal egg count (FEC) by a factor (20) [27, 32]. The formula are shown below;

Number in one gram =  $\frac{Number in two chambers}{0.3}$  x dilution factor

 $Dilution factor = \frac{Total \ volume \ of \ suspension \ in \ ml}{Total \ volume \ of \ faeces}$ 

## Percentage Reduction of Egg Per Gram (EPG) and Deparasitisation of Adult worm

Anthelmintic efficacy of CMSBE, EA fraction and AQ fraction of *P. biglobosa* were assessed by counting the eggs and worms in the treated animals and comparing with counts from the untreated control WAD goats. The percentage efficacy (Deparasitisation) of adult parasites and Percent reduction in faecal egg count were calculated using the following formulae [33].

% Efficacy = 
$$\frac{N-n}{N} X 100$$
, Where

N= Mean number of *H. contortus* in control (untreated) animals

n = Mean number of *H. contortus* in treated animals.

Percent reduction in faecal egg count will be computed by the following formula:

% Reduction = 
$$\frac{\text{Mean EPG on day 0} - \text{Mean EPG on day 16}}{\text{Mean EPG on day 0}}$$

#### Post Mortem Examination of Animals after in vivo Study

One animal in control groups (un-treatment - group A and standard drug – group B) and treatment groups (C, D, E and F) each was slaughtered on day 16 post-treatment for total worm count. The gastrointestinal tract was observed for presence of adult *H. contortus*. Also, the abomasal contents were collected and the walls of the abomasums were washed with water. The washings and the contents of the large bowel were combined and wash through sieves of appropriate aperture for worm counts [34].

#### **Statistical Analyses**

The data were computed to determine the means and standard deviation. Data obtained for egg and adult counts were expressed as mean±SEM. T- test and One Way Analysis of Variance (ANOVA) followed by Turkey's post hoc test were used where necessary.

Value of P < 0.05 was considered significant. GraphPad Instat version 3.05 Windows from Graphpad Software (2000), San Diego, California USA (www.graphpad.com) was used to analyze the data.

# **RESULTS AND DISCUSSION**

The solvent partitioning of 284.28 g of CMSBE was negligible for n- Hexane but yielded 160.53 g and 97.15g for aqueous (AQ) and Ethyl acetate (EA) fractions respectively (Table 1). The remaining 26.6 g portion was discarded as residue. The colour and percentage yield of the plant material are shown in Table 1. Generally, partitioning with water resulted in the highest quantity of crude extract and followed by ethyl acetate. The phytochemical constituents present in partitioning of crude methanol stem bark extract of *P. biglobosa* are shown in Table 2. In comparison, both EA and AQ fractions contained anthraquinones, cardiac glycosides, phlobatannins, saponins and tannins (condensed and hydrolysable). The EA fraction alone revealed the presence of alkaloids, flavonoids, oil and sterol/steroids while AQ fraction alone revealed the presence glycosides, reducing sugar and terpenoid. The saponins and tannins (condensed and hydrolysable) in EA fraction were higher when compared to AQ fraction. However, anthraquinones was higher in AQ fraction, all without significant difference.

The effects of CMSBE and its fraction on egg per gram faeces (EPG) are showed in Table 3. Significant (P<0.05) reduction in EPG was observed in all extract-treated groups in all the dose level used during the study (Table 3). A graded dose response in EPG reduction was recorded in group D which was more effective than observed in other groups. Although, generally, a significant (P<0.05) decrease in EPG was observed in group B (positive control), D and E on day 4 when compared to other groups as shown in Table 3. The decrease in EPG in group B, D and E also continued from 4, 8, 12 and 16 days without significant different (P>0.05) but the decrease in group C and F for 4, 8, 12 and 16 days was significant (P<0.05) different (Table 3). The untreated group (group A) showed no reduction in EPG, rather there was slight increase in EPG from day 0 to 4 and 8 days with fluctuations on the 12th and 16th days (Table 3). In all the days of faecal eggs examinations for EPG in group A, there was no significant difference (P>0.05) as shown in Table 3. However, the faecal eggs examination for EPG on day 0 of group B, C, D, E and F differ significantly from different days (4, 8, 12 and 16 days). Therefore, the best in vivo anthelmintic activity based on faecal egg count reduction test (FECRT was exhibited by group D followed in descending order by group B, E, C and F at day 16 post treatment (Table 4).

The highest percentage (99.4%) deparasitisation of adult worms in WAD goats was found in group E administered with EA portion of CMSBE of *P. biglobosa* as shown in Table 5. This was followed in a decreasing order by group D, B, C and F with percentage deparasitisation of 93.1, 92.3, 76.8 and 30.1%, respectively.

CMSBE partitioning	Initial weight (g)	Final weight(g)	Colour of extract	Percentage yield (%)
Aqueous fraction	284.28	160.53	Dark brown	56.47
Ethyl acetate (EA)	284.28	97.15	Light brown	34.17
n-Hexane	284.28	0	No colour change	0
Residue	284.28	26.6	Dark browm	9.36

 Table 1. Percentage Yields of Stem Bark Extracts of P. biglobosa of Crude Methanol

 Partitions

Table 2. Qualitative Phytochemical Screening of Partitioning of Crude Methanol Stem
Bark Extracts of Parkia biglobosa

Chemical	Test methods	Crude Metanol	Ethyl acetate	Aqueous
constituents	i est methous	extract	fraction	fraction
Alkaloids	Mayer's test	+++	++	machon
maionas	Wagner's	+++	+++	—
Anthraquinones	Bontrager's test	+++	++	_ +++
Cardiac	Keller-Kiliani test	+	+	+
Glycosides	Rener Rindin test	I	Ĩ	I
Flavonoids	NaoH test	++	++	
Glycosides	Benedict's test,	++		_ ++
Gijeobideb	Ferric chloride	++	-	++
	test	1 1	-	1 1
Oil	Filter paper test	++	+	
Protein	Millon reagent		Ĩ	—
I I Otem	test	_	—	_
	Biuret test			
Phlobatannins	Hcl test	_ ++	_ +	- +
Reducing Sugar	Fehling test	++	I	+++
Saponins	Frothing test	+++	_ +++	++
Starch	Iodine test			
Sterols and	Conc H <sub>2</sub> So <sub>4</sub> test	_ ++	_ ++	_
Steroids				_
Tannin	Ferric chloride	+++	+++	++
(Condensed)	test			
Tannin	Ferric chloride	++	+++	++
(Hydrolysable)	test			
Terpenoid	Salkowski test	++		++
Triterpenoids	Salkowski test		_	
· · · ·	ev = -Absent + Present +			

Key= - Absent, + Present, ++ Very present, +++ much present

Experimental	<b>Pre-treatment</b>		Post-tre	atment	
Groups (mg/kg)	Day 0	Day 4	Day 8	Day 12	<b>Day 16</b>
Α	14593.33±40.71ª	15026.67±43.54ª	$16460 \pm 14.7^{a}$	14520±48.00 <sup>a</sup>	14895±26.00 <sup>a</sup>
В	13793.33±90.35ª	500±90.35 <sup>b</sup>	$413.33{\pm}16.84^{b}$	413.33±89.69 <sup>b</sup>	273.33±81.92 <sup>b</sup>
С	13773.33±11.27ª	3260±27.33°	1726.67±27.41 <sup>b</sup>	1486.67±22.01°	$1593.33 \pm 26.40^{bc}$
D	13573.33±71.83ª	$450{\pm}39.74^{bd}$	$200 \pm 52.92^{b}$	$180.00{\pm}20.00^{bd}$	$180.00{\pm}20.00^{bd}$
Ε	$14666.67{\pm}19.50^{a}$	933.33±95.16 <sup>be</sup>	$486.67 \pm 48.96^{b}$	$446.67 \pm 46.97^{be}$	$420{\pm}49.67^{bce}$
$\mathbf{F}$	13760±67.03ª	$2433.33{\pm}53.24^{\rm f}$	$2373.33{\pm}13.00^{\rm bf}$	$3726.67{\pm}35.19^{\rm f}$	6340±11.40 <sup>g</sup>

**Table 3.** Effects of CMSBE, Ethyl Acetate Portion and Aqueous Portion of P. biglobosaand Standard Drug (Albendazole) on Mean EPG in WAD Goats Infected with L3 of H.contortus

The mean with different superscript alphabet in the same column and row are statistically significant different (P<0.05)

#### Keys :

A<sup>+</sup> = Untreated control - distilled water (DW) 5ml/kg B<sup>\*</sup> = Treated control- Albendazone (ABZ) 6.25 mg/kg C= CMSBE of *P. biglobosa* (1000mg/kg) D= CMSBE of *P. biglobosa* (2000mg/kg) E= EA fraction of CMSBE (1000mg/kg) F= AQ fraction of CMSBE (1000mg/kg \*MCD as recommended by manufacturer <sup>+</sup>MCV <sup>+</sup>MCV

**Table 4.** Percentage Reduction of EPG from WAD Goats Infected with 2500 L<sub>3</sub> of H. contortus and Orally Treated with CMESb, EA Portion and AQ Portion of P. biglobosa for Three Consecutive Days.

Group	<b>Pre-treatment</b>	Post-treatment			Percentage	
	Day 0	Day 4	Day 8	Day 12	Day 16	change
Α	0	-3	-13	1	-2	
B	0	96.4	97.00	97.00	98.02	97.11 <sup>a</sup>
С	0	76.33	87.46	89.21	88.43	85.36 <sup>a</sup>
D	0	96.68	98.53	98.67	98.67	98.14 <sup>a</sup>
Ε	0	93.64	96.68	96.95	97.14	96.11 <sup>a</sup>
F	0	82.32	82.75	72.92	53.92	72.98 <sup>b</sup>

F = 10.438 = (MStreatment/MSresidual). a, b differ significantly (p<0.05) from one another

#### Keys

 $A^+$  = Untreated control - distilled water (DW) 5ml/kg  $B^*$  = Treated control- Albendazone (ABZ) 6.25 mg/kg C= CMESb of *P. biglobosa* (1000mg/kg)

D = CMESb of P. biglobosa (2000mg/kg)

E = EA fraction of CMSBE (1000mg/kg)

F = AQ fraction of CMSBE (1000mg/kg)

\*MCD as recommended by manufacturer

+MCV

Experimental group (mg/ml)	Total adult count	% Deparasitisation
Α	362	0
В	28	92.3
С	84	76.8
D	25	93.1
Ε	2	99.4
F	253	30.1
Keys		

 Table 5. Percentage Deparasitization of Adult H. contortus from Infected WAD Goats

 Administered Orally with CMSBE, EA Portion and AQ Portion of P. biglobosa for 3

 Consecutive Days

A = Untreated control - distilled water (DW) 5ml/kg

B = Treated control- Albendazone (ABZ) 6.25 mg/kg

C = CMESb of P. biglobosa (1000mg/kg)

D= CMESb of P. biglobosa (2000mg/kg)

E= EA fraction of CMSBE (1000mg/kg)

F=AQ fraction of CMSBE (1000mg/kg

Medicinal plants offered a great prospect for the development of novel chemotherapeutic agents that are essential for the management of various diseases in food animals and human. In this study, the result of qualitative phytochemicals screening of CMSBE of *P. biglobosa* showed the present of alkaloid, anthraquinones, cardiac glycosides, glycosides, flavonoids, oils, phlobatannins, reducing sugar, saponins, sterols/steroids, tannin (condensed and hydrolysable) and terpenoids. This result was similar to the findings of Ezekwe *et al.* [35] in methanol stem bark extract of *P. biglobosa*. Millogo Kone *et al.* [36] also reported the presence of saponins, Glycosides, tannins and other phenolics with trace quantity of alkaloids while Banwo *et al.* [37] confirmed the same. The report of Builder *et al.* [17] differed slightly from this result and the previous finding of Banwo *et al.* [36-37] due to absence of alkaloids from the methanol stem bark extracts. Thus, the difference between the phytochemical constituents 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.

Generally, partitioning with water resulted in the highest quantity of crude extract, while n-Hexane yielded nothing. This high percentage yielded by water might be due to the high polarity associated with it and to some extent some less polar compounds were extracted [38]. N-Hexane is non-polar and this probably account for its inability to extract those biologically active compounds. The general trend of the yield obtained in ascending order was n-hexane < Ethyl acetate < water. Ethyl acetate and water are polar solvents; this explained the fact that the secondary metabolites extracted from this plant using these solvents are polar compounds. This showed that like solvents dissolved like [11]. Although, water is more polar and have tendency to extract hydrosoluble compounds, substances like Ethyl acetate in addition to hydrosoluble extracted, have tendency to extract lipid substances, alkaloids, and phenols [39]. The semi polar nature of the ethyl acetate which can extract both apolar and polar secondary metabolites explained why the Ethyl acetate fraction extracted alkaloid and oil that could not be extracted using water. Thus the extraction of active principles from the medicinal plants for pharmacological

evaluation was to some extent dependent on the polarity of the solvents used in the extraction and partitioning.

In this study, ABZ, CMSBE of P. biglobosa and EA and AQ fractions were tested in vivo for anthelmintic activity and they were found to be a very potent anthelmintic. They produced reduction in faecal egg count/egg per gram (FEC/EPG) and deparasitisation as observed from daily faecal analysis and adult worm counts postmortem in WAD goats. All the treatment based on ABZ, CMSBE, and EA and AQ fractions of P. biglobosa exhibited high reduction in FEC of WAD goats infected with H. contortus. The 96.4% drop in the faecal EPG count in the group B treated with ABZ (6.25 mg/kg) on 4th day, clearly indicates the high anthelmintic effectiveness in goats. The groups C, D, E and F had 76.33%, 96.68%, 93.64% and 82.32% reduction in the faecal EPG respectively on 4th day. This also indicated the effectiveness of the extracts. On 16th day post treatment, FEC had reduced to 98.02% for ABZ and 88.02%, 98.67%, 97.14% and 53.93% for groups C, D, E and F, respectively. It could be stated that ABZ in group B, CMSBE in group D and EA in group E were effective against H. contortus and that there was no resistance of this parasite against the ABZ and the extract used as well, since the FEC reduction was greater than 90%. The criteria for evaluating the degree of efficacy of an anthelmintic, was in accordance to Bliss et al. [40] and recommendation of the World Association for the Advancement of Veterinary Parasitology (WAAVP) which say, anthelmintic resistance is present if percentage reduction of egg counts is less than 90% [41].

This result agreed with that of Naandam and Iddrisu [42] who reported 80.4% reduction in ova counts in infected sheep administered with 4 mL of boiled pods extract of P. biglobosa after one month. It was observed that there was an increase reduction in faecal EPG counts in group F between days 4 and 8 but decline on days 12 and 16 as faecal EPG counts was greater than days 4 and 8. This suggested that the aqueous extract as a drug may be acting over a reasonable number of days as recorded by Birkett [43], who confirmed that after a single dose, drug concentration falls with time. The increase in EPG between days 12 and 16 might be due to the fact that active ingredients such as tannins and saponins lost their potency as secondary metabolites with time, which led to an increase in the population of worms. The criteria of WAAVP were fulfilled by ABZ, CMSBE (2000 mg/kg), and EA fraction (1000 mg/kg) which had 98.02%, 98.67% and 97.14% reduction of EPG of faeces on 16th day respectively. These results agreed with the results of Naandam and Iddrisu [42] who reported 93.8% reduction in ova counts in infected sheep administered with 4 ml of pounded and soaked pods of P. biglobosa after one month. It can then be said that the efficacy of CMSBE administered to goats at 2000 mg/kg and EA fraction at 1000 mg/kg are comparable to that of ABZ (the conventional anthelmintic) at a dose rate of 6.25 mg/kg.

The result of anthelmintic study also indicated that EA fraction of *P. biglobosa* produced 99.4% deparasitisation followed by CMSBE and then lastly AQ portion. It is worthy to note that the percentage deparasitisation of EA portion was even more than the standard drug ABZ that has 92.3% deparasitisation. This is an indication that the extracts showed high *in vivo* anthelmintic efficacy than the ABZ. It was then considered that CMSBE (at 2000mg/kg and 1000mg/kg) and EA fraction of *P biglobosa* at 1000 mg/kg administered to infected goats were effective. The criteria of Githiori *et al.* [44] was also established, who reported that the efficacy of the plant extracts would be biologically significant if a reduction in total worm count (TWC) above 70% occurred.

The active principle(s) in extract(s) responsible for this anthelmintic activity might be individual phytochemical constituents as detected during phytochemical screening or a number of them working in synergy. The higher effect, according to Wabo et al. [45] could be due to secondary metabolites such as tannins, flavonoids, polyphenols and alkaloids. This assertion was made from their findings on in vitro study on leaf extracts of Ageratum conizoides on against Heligmosomoides polygyrus- the nematode of rat. These compounds created unfavorable conditions to the survival of the parasites. Sina and Traoré [46] had reported that the bark, leaves and pod husks of P. biglobosa was rich in tannins, which Max et al. and Octhere and Naandam [47-48] suggested had direct toxicity action on worms in drench sheep. Condensed tannins-containing forages have the potential to help control anthelmintics resistant gastrointestinal parasites. They have been shown to decrease EPG in sheep and goats and may decrease hatching rate and larval development in faeces [49]). This is also in agreement with Max et al. [50], who showed that there is an effect of tanniferous browsers meal on faecal egg counts and internal worm burdens. The tannins contained in plants have been reported to posses antiviral [51], antibacterial [52] and anthelmintic [53-55] properties.

The pharmacological basis of the treatment of helminthes possibly involves disruption of the energy processes of the helminthes. The benzimidazoles/probenzimidazoles (e.g. albendazole, mebendazole, thiabendazole, fenbedazole, and flubendazole) act by interferring with polymerization of microtubules [56]. These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation [57].

The modes of action of anthelmintics are many, reflecting the natural differences in the physiology of the parasites and its potential host. It has been firmly established that one of the hallmark effects of any anthelmintic is the destruction of the worm's cuticle. This is due to the fact that the tegument and/or cuticular structures are the primary parasite-host interface vital for absorption of nutrients and perception of the surrounding micro-environment provided by the host [58-59].

# CONCLUSION

This study has established that the CMSBE of *P. biglobosa* and its EA fraction extract have potentials anthelmintic activities against experimental *H. contortus* infection in WAD goats at doses 2000mg/kg and 1000mg/kg of goats respectively. It is worthy to note that the percentage deparasitisation (99.4%) of EA fraction was more than standard drug ABZ that has 92.3% deparasitisation. This is an indication that the extracts showed high *in vivo* anthelmintic potentials than the ABZ. The high *in vivo* anthelmintic exhibited in this study may be attributed to the presence of secondary metabolites in the extracts. It is therefore recommended to carry out a further research on a larger population size of goats.

**Acknowledgement.** We would like to appreciate the technical staff of STEB –B centre for Biotechnology Laboratory, Federal University of Technology (FUT) Minna, Biochemistry Laboratory, FUT Minna and Biological Garden of Centre for Preliminary and Extral-Mural Studies, FUT, Minna for their technical supports.

**Conflict of Interest.** The authors declare no potential conflicts of interest with regard to this research, authorship, and/or publication of this article.

Authorship Contributions. Concept: J.J., J.A., I.O., Design: J.J., J.A., I.O., Data Collection or Processing: J.J., J.A., O.O., Analysis or Interpretation: J.J., O.O., J.A., Literature Search: J.J., O.O., I.O., Writing: J.J.

Financial Disclosure. This research received no grant from any funding agency/sector.

#### REFERENCES

- [1] Sahlu, T., Dawson, L.J., Gipson, T.A., Hart, S.P., Merkel, R.C. (2009): ASAS Centennial Paper: Impact of animal science research on United States goat production and predictions for the future, Journal of Animal Science 87: 400-418.
- [2] Tariq, K.A., Chishti, M.Z., 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.
- [3] Owhoeli, O., Elele, K., Gboeloh, L.B. (2014): Prevalence of Gastrointestinal Helminths in Exotic and Indigenous Goats Slaughtered in Selected Abattoirs in Port Harcourt, South-South, Nigeria. Chinese Journal of Biology 435913: 8pp.
- [4] Maciel, M.V., Morais, S.M., Bevilaqua, C.M.L., Camurca-Vasconcelos, A.L.F., Costa, C.T. C., Castro, C.M.S. (2006): Ovicidal and larvicidal activity of *Meliaazedarach* extracts on *Haemonchus contortus*. Veterinary Parasitology 140:98-104.
- [5] 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.
- [6] Sawleha, Q., Dixit, A.K., Pooja, D. (2010): Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants. Veterinary World 3(11):515-518.
- [7] Waller, P.J. (2006): Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology 126: 277-279.
- [8] Soetan, K.O., Aiyelaagbe, O.O. (2009): The Need for Bioactivity-Safety Evaluation and Conservation of Medicinal Plants- A Review. Available online at http://www.academicjournals.org/JMPR. Journal of Medical Plants Research 3(5): 324-328.
- [9] Sandoval-Castro, C.A., Torres-Acosta, J.F.J., Hosteb, H., Salemd, A.Z.M., Chan-Péreza, J.I. (2012): Using plant bioactive materials to control gastrointestinal tract helminths in livestock. Journal of Animal Feed Science and Technology 176: 192-201.
- [10] Athanasiadou, S., Githiori, J., Kyriazakis, I. (2007): Medicinal plants for helminth parasitecontrol: Facts and fiction. Animal Consortium 1: 1392-1400.
- [11] Udobi, C.E., Onaolapo, J.A. (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: 338-344.
- [12] Tokoudagba, J.M., Augera, C., Bréant, L., N'Gom, S., Chabert, P., Idris-Khodja, N., Schini-Kerth, V.B. (2010): Procyanidin-rich fractions from Parkia biglobosa (Mimosaceae) leaves cause redox-sensitive endothelium-dependent relaxation involving NO and EDHF in porcine coronary artery. Journal of Ethnopharmacology 132: 246–250.
- [13] Etuk, E.U., Bello, S.O., Isezuo, S.A., Mohammed, B.J. (2010): Ethonobotanical survey of medicinal plants used for the treatment of diabetes mellitus in the north western region of Nigeria. Asian Journal of Experimental Biology Science 2(1):55-59.
- [14] Gronhaug, T.E., Glaeserud, S., Skogsrud, M., Ballo, N., Bah, S., Diallo, D., Paulsen, B.S. (2008): Ethnopharmacological survey of six medicinal plants from Mali. West African Journal of Ethnopharmacology 4:4-26.
- [15] Tijani, A.Y., Okhale, S.E., Salawu, T.A., Onigbanjo, H.O., Obianodo, L.A., Akingbasote, J.A., Emeje, M. (2009): Anti diarrheal and antibacterial properties of crude aqueous stem bark extract and fractions of P. biglobosa (Jacq) R.Br Ex G. Don. African Journal of Pharmacy and Pharmacology 7:347-353.

- [16] Soetan, K.O., Lasisi, O.T., Agboluaje, A.K. (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.
- [17] Builders, M.I., Isichie, C.O., Aguiyi, J.C. (2012): Toxicity Studies of the Extracts of *Parkia biglobosa* Stem Bark in Rats. British Journal of Pharmaceutical Research 2(1): 1-16.
- [18] Emmanuel, O.A., David, A.A., Olayinka, A.A., Mobolaji, F.A., Matthew, O.O., Anthony, I.O. (2013): Preliminary Phytochemical Screening and Antibacterial Properties of Crude Stem Bark Extracts and Fractions of *Parkia biglobosa* (Jacq.) Molecules 18 (7): 8485-8499.
- [19] Lorke, D. (1983): A new approach to practical acute toxicity testing. Arch. Toxicology 54:275-287.
- [20] Ibrahim, M.A. (1984): Evaluation of the activities of some West African Traditional anthelmintic herbs against *Nippostrongylus brasiliensis in* rats. *M*.Sc. Thesis, Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria pp. 116.
- [21] Loomis, T.A. (1978): Essentials of Toxicology. Third edition. Lea and Fibiger, Philadephia pp 198.
- [22] Kimani, D., Kareru, P.G., Njonge, F.K., Kutima, H.L., Nyagah, G.C., Rechab, S.O., Wamburu, R.W., Karanja, J.M. (2014): Control of gastro-intestinal nematodes in ruminants using plant extracts. Scientific conference proceeding pp.193-198.
- [23] Evans, W.C. (2002): Trease and Evans' Pharmacology 15<sup>th</sup> Edition, W.B. Saunders, New York pp. 221-393.
- [24] Edeoga, H.O., Okwu, D.E., Baebie, B.O. (2005): Phytochemical constituents of some Nigerian medicinal plants: African Journal of Biotechnology 4 (7):685-688.
- [25] Makun, H.J., Ajanusi, O.J., Lakpini, C.A.M., Ehoche, O.W., Rekwot, P. I. (2008): Response of Red Sokoto and Sahelian Goats to Trickle *Haemonchus contortus* Infection. Journal of Biological Sciences 8: 753-759.
- [26] Soulsby, E.J.L. (1986): Helminths, *Arthropods and protozoa of domesticated Animals*, 7<sup>th</sup> *Edition Bailliere Tindall, London* pp. 119-218.
- [27] Hansen, J., Perry, B. (1994): The Epidemiology, Diagnosis and control of Helminth parasites of ruminants. *International Livestock Center for Africa*, <u>Addis Ababa Ethiopis</u> pp: 90-100.
- [28] Anaeto, M., Tayo, G.O., Chioma, G.O., Afolabi, A.A. (2009): Comparative study of Albendazole and Papaya seed in the control of Gastrointestinal Nematodes in Goats. Journal of life and physical sciences, acta SATECH 3(1): 25-28.
- [29] Chiejina, S.N., Behnke, J.M., Musongong, G.A., Nnadi, P.A, Ngongeh, L.A. (2010): Resistance and resilience of West African Dwarf goats of the Nigerian savanna zone exposed to experimental escalating primary and challenge infections with Haemonchus contortus. Journal of Veterinary Parasitology 171: 81–90.
- [30] Lucas, A.N., Amaechi, O. (2015): Comparative Response of the West African Dwarf Goats to Experimental Infections with Red Sokoto and West African Dwarf Goat Isolates of *Haemonchus contortus*. Journal of Pathogens 728210: 6.
- [31] Worku, M., Franco, R., Miller, J. H. (2009): Evaluation of the Activity of Plant Extracts in Boer Goats. American Journal of Animal and Veterinary Sciences 4 (4): 72-79.
- [32] Sujon, M.A., Mostofa, M., Jahan, M.S., Das, A.R., Rob, S. (2008): Studies On Medicinal Plants Against Gastroinstestinal Nematodes Of Goats. Bangladesh Journal of Veterinary Medicine 6 (2): 179–183.
- [33] Arundel, J.H. (1985): Veterinary anthelmintics. University of Sydney Postgraduate Foundation in Veterinary Science, New South Wales, Australia. Journal of Veterinary, Revised 26: 118.
- [34] MAFF (Ministry of Agriculture, Fisheries and Food). (1986): Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London.
- [35] Ezekwe, C.I., Anaya, C.A., Okechukwu, P.C.U. (2013): Effects of Methanol Extract of *Parkia biglobosa* Stem Bark on the Liver and Kidney Functions of Albino Rats. Global Journal of Biotechnology and Biochemistry 8 (2):40-50.

- [36] Millogo-Kone, H., Guissou, I.P., Nacoulma, O., Traore, A.S. (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.
- [37] Banwo, G O., 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 1: 16-22.
- [38] Adeyemi, O.O., Okpo, S.O., Young-Nwafor, C.C. (2009): The relaxant activity of the methanolic extract of *Acanthus montanus* on intestinal smooth muscles. Journal of Ethnopharmacology 68(1-3): 169-173.
- [39] Ciulei, I. (1982): Practical Manuals on the Industrial Utilization of Chemical and Aromatic Plants.Methodology for Analysis of Vegetable Drugs.1<sup>st</sup> edition, Ministry of Chemical Industry, Bucharest pp. 67.
- [40] Bliss, D.H., Moore, R.D., Kvasnicka, W.G. (2008): Parasite resistance in U.S. cattle. American Association of Bovine Practitioners 41: 109-114.
- [41] Coles, G.C., Bauer, F.H.M., Borgsteede, S., Greerts, S., Klei, T.R., Taylor, M.A., Waller, P.J. (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: 35-44.
- [42] Naandam, J., Iddrisu, R. (2010): Effect of *Parkia biglobosa* (Dawadawa) Pod extracts on strongyle ova in sheep. Animal Research International 7(3):1267–1273.
- [43] Birkett, D.J. (1995): Pharmacokinetics made easy: 10 Pharmacodynamics-the concentrationeffect relationship. Australian Prescription 18:102-4.
- [44] Githiori, J.B. (2004): Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by Pastoralists and small holder farmers in Kenya. Doctoral thesis ISSN 1401-6257.
- [45] Wabo, P.J., Tankoua, O.F., Yondo, J., Komtangi, M.C., Mbida, M., Bilong Bilong, C.F. (2011): The *in vitro* effects of aqueous and ethanolic extract of leaves of Ageratum conizoides (Asteraceae) on three life cycle stages of the parasitic Nematode *Heligmosomoides bakeri* (Nematoda: Heligmosomatidae). Journal of Veterinary Medicine International 140293: 1-5.
- [46] Sina, S., Traoré, S.A. Parkia biglobosa (Jacq.) R.Br.exG.Don. In: Oyen, I.P.A, Lemmens R.H.M.J. (2002): (Editors), PROTA (Plant Resources of *Tropical Africa / Ressources végétales de l'Afrique tropicale*), Wageningen, The Netherlands 1-200.
- [47] Max R.A., Buttery, P.J., Wakelin, D., Kimambo, A.E., Kassuku, A.A., Mtenga, I.A. (2003): The potential of controlling gastrointestinal parasitic infections in tropical small ruminants using plants high in tannins or extracts from them. In: Proceedings of the Third DFID Livestock Production Programme Link Project (R7798) Workshop for Small Ruminant Keepers. Izaak Walton Inn, Embu, Kenya, 4 – 7 February 2003.
- [48] Octhere, G.V., Naandam, J. (2015): Effect of pounded dawadawa (*Parkia biglobosa*) pod husk extract on strongyle in west african dwarf (WAD) goats. UDS International Journal of Development [UDSIJD] 1 (1): 11-16.
- [49] Min, B.R., Hart, S.P. (2003): Tannins for suppression of internal parasites. Langston University, Langston. Pub. American society of animal science. Journal of Animal Science 81(2): 102-109.
- [50] Max, R.A., Wakelin, D., Dawson, J., Kimambo, A. E., Mtenga, L.A., Buttery, P.J. (2007). Effects of tanniniferous browse meal on nematode faecal egg counts and internal parasite burdens in sheep and goats. University of Nottingham. Journal of Animal Science 37(2): 97-104.
- [51] Cheng, H.Y., Lin, C.C., Lin, T.C. (2002): Antiherpes simplex virus type 2 activity of casuarinin from the bark of Terminalia arjuna Linn. Antiviral Resource 55: 447–455.
- [52] Perumal, S.R., Ignacimuthu, S., Sen, A. (1998): Screening of 34 Indian medicinal plants for antibacterial properties. Journal Ethanopharmacology 62: 173–182.

- [53] Paolini, V., Bergeaud, J.P., 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.
- [54] Paolini, V., De La Farge, F., Prevot, F., Dorchies P.H., 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: 277–283.
- [55] Ademola, I.O., Fegbemi, B.O., Idowu, S.O. (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.
- [56] Harder A. (2002): Chemotherapeutic approaches to nematodes: current knowledge and outlook. Parasitology Research 88: 272-277.
- [57] Susan, S. (2016): Understanding anthelmintics. Maryland Small Ruminant 1-14.
- [58] Williams, S., Botros, S., Ismail, M., Farghally, A., Day, T.A., Bennett, J.L. (2001): Praziquantel-induced tegumental damage *in vitro* is diminished in schistosomes derived from Praziquantel-resistant infection. Parasitology 122: 63-66.
- [59] Xiao, S.H., Guo, J., Chollet, J., Wu, J.T., Tarma, M., Utizinger, J. (2004): Effect of artemether on *Schistosoma mansoni:* dose-efficacy relationship, and change in worm morphology and histopatholoy. Chineese Journal of Parasitological Diseases 22: 148-153.