

RESEARCH

Open Access



Effects of *Vernonia amygdalina* methanol leaf extract and fractions on *Ascaridia galli* in experimentally infected birds with regard to its pathological effect

Khadijat Abidemi Gasaliyu^{1*} , Oseka Joseph Ajanusi¹, Mohammed Musa Suleiman², Sani Dahiru², Kuburat Hadiza Yusuf¹, Stephen Kyari^{1,4}, Matthew Ogwiji¹ and Ochuko Orakpoghenor³

Abstract

Background: Helminthiasis, usually a chronic problem in poultry, is the major cause of economic losses in the poultry industry. This study was undertaken with the aim of evaluating the in vitro and in vivo anthelmintic effects of *Vernonia amygdalina* methanol leaf extract and fractions on *Ascaridia galli* in experimentally infected birds. Standard protocols for extraction, phytochemical screening, isolation and infection of parasite and histological examination were followed.

Result: At 50 mg/ml albendazole (ALB) caused the highest inhibition of embryonation (97.3%) followed by crude methanol extract (CME) (94.5%), ethyl acetate fraction (EAF) (81.3%) and butanol fraction (BF) (80.3%). The lowest faecal egg counts (FEC) and highest FEC at 21 days post-treatment were observed in the group treated with albendazole (99.2%) and CME at 800 mg/kg (96.9%). Deparasitization at 200 mg/kg caused by ALB was (93.8%), while at 200, 400 and 800 mg/kg, CME was (77.5%, 87.5% and 91.86%), BF was (75.51%, 79.61% and 85.73%) and EAF was (73.48%, 75.5% and 81.63%), respectively. At post-mortem, broiler chickens infected with *A. galli* and treated with CME, BF and EAF at 200 mg/kg had desquamation of the villi while at 400 and 800 mg/kg treatments, the intestines were intact.

Conclusion: The extract and fractions of *V. amygdalina* were found to possess anthelmintic activity against *A. galli* due to the inhibition of embryonation of eggs and the reduction in faecal egg count; further research is required to fully ascertain their mechanism of action of the extract.

Keywords: Anthelmintic, Histopathology, *Vernonia amygdalina*, *Ascaridia galli*, Birds

Background

Poultry production is an important means of providing high-quality protein for human consumption and contributes about 30% of all animal protein consumed globally (FAO 2010). Poultry reared in rural scavenging system face various hindrances among which helminthiasis plays a vital role (Fakae and Paul-Abiade 2003). Studies

conducted in different parts of the world indicated that the proportion of chicken infection with gastrointestinal helminths is high; therefore, helminths are considered to be an important cause of ill health and reduction in poultry productivity (Ajala et al. 2007; Ogbaje et al. 2012; Idika et al. 2016; Ola-Fadunsi et al. 2019). Helminthiasis is a chronic problem in the poultry industry and accounts for economic losses (Newbold et al. 2017). *Ascaridia galli* is the largest, most prevalent and one of the most pathogenic nematodes of birds. Infection may cause reduction in growth rate, weight loss, sometimes serious illness,

*Correspondence: khadeegaz2015@gmail.com

¹ Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria

Full list of author information is available at the end of the article

economic losses and occasionally, mortality (Yousfi et al. 2013; Naphade 2014; Silva et al. 2015; Sahu and Sinha 2016). There are reports of emerging resistance to some anthelmintics (Coles et al. 1998) due to the use, overuse and misuse of the drugs in the treatment and prevention of parasitic infections (Douglass et al. 2015; Lawal et al. 2015). Ethno-veterinary medicine (EVM) as an alternative for controlling both internal and external parasites in livestock production systems involves the use of indigenous beliefs, knowledge, skills, methods and practices pertaining to the health care of animals (Mathius-Mundy and McCorkle 2004). *V. amygdalina* commonly called bitter leaf (English) due to its bitter taste was named after an English Botanist William Vernon (Alara et al. 2017). In Nigeria, it is known as 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onuigbo' in Igbo, 'Ityuna' in Tiv, 'Oriwo' in Edo and 'Shuwaka' in Hausa (Farombi and Owioye 2011). So, the aim of the study was to evaluate the anthelmintic and histopathological efficacy of the methanol leaf extract and fractions of *V. amygdalina* against experimental *A. galli* infection in broilers and assess the histopathological lesions in the extracts-treated birds. The traditional use of the plant as an anthelmintic necessitated this study.

Methods

Ethical clearance

Ethical approval for the use of animal for this study was approved by the Ahmadu Bello University, Committee on Animal Use and Care (ABUCAUC/2021/042), ABU Zaria.

Experimental location

This study was carried out in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Zaria.

Collection, identification and preparation of *V. amygdalina* leaves

Fresh *V. amygdalina* leaves and flowers were collected from the Botanical Garden of Ahmadu Bello University, Zaria, between the months of October and November 2019. The Sample was taken to the Herbarium of the Department of Botany, ABU Zaria and compared with voucher specimens kept at the Herbarium and a voucher number (ABU07425) corresponding to that specimen was allocated. The leaves were then dried under the shade at room temperature (Abdalla et al. 2012). The dried leaves were made into powder using mortar and pestle. The powdered leaf material was weighed, poured into a polythene bag and kept at room temperature.

Extraction and solvent partitioning

The extraction of the *V. amygdalina* leaves was carried out at the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, ABU Zaria according to the method described by (Handa et al. 2008). Briefly, about 3.5 kg of the powdered leaves of *V. amygdalina* was extracted with 10.5 L of absolute methanol by maceration for 48 h and filtered. The procedure was repeated three times to exhaustively extract the constituents of the plant materials. Thereafter, the extract was filtered. The filtrates were pooled together and solvent was evaporated *in vacuo* at 40 °C using a rotary evaporator to obtain the crude methanol extract (CME). The CME was divided into two portions: One portion for the partitioning while the other portion was kept for the treatment during *in vivo* studies. One hundred and fifty grams (150 g) of the solid crude methanol extract (CME) was dissolved in 1.5 L of distilled water to form an aqueous extract with 1.5L of Butanol added and exhaustively mixed together. The suspension was sieved through a filter paper to obtain the Butanol fraction (BF). 1.5 L of ethyl acetate was added to the aqueous extract and exhaustively mixed together, sieved through a filter paper to obtain the Ethyl acetate fraction (EAF). Both fractions were concentrated to a semi gel-like material by allowing the solvent to evaporate at room temperature. The process was done for hexane (HF) fractions also.

Phytochemical screening

The phytochemical screening of the plant extract and fractions were carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU Zaria. Phytochemical test was carried out on the crude methanol extract, hexane, butanol, ethyl acetate fractions according to the methods described by Trease and Evans (1996).

Test for carbohydrate

Molisch's test

Three drops of Molisch's reagent were added to about 0.1 g of each crude extract and the fractions (hexane, ethyl acetate and butanol), each in a test tube, followed by addition of concentrated sulphuric and proper mixing. The mixtures were allowed to stand for two minutes. The formation of a reddish colour ring at the interface indicated the presence of carbohydrate.

Test for flavonoids

Shinoda test (Stephen et al. 2021)

One hundred microgram of the extract and fractions were each dissolved in 2 ml of methanol and pieces of magnesium chips were added, followed by addition of 3

drops of concentrated hydrochloric acid. The formation of a pink, orange or red to purple colouration indicated the presence of flavonoids.

Sodium hydroxide test (Stephen et al. 2021)

Two drops of 10% sodium hydroxide solution were added to 0.1 g of the extracts (CME) and each of the fractions (hexane, ethylacetate, butanol). Appearance of yellow colouration indicated the presence of flavonoids.

Test for saponin

Frothing test (Stephen et al. 2021)

Ten ml of distilled water was added to 0.1 g of the extract and each fraction (hexane, ethylacetate, butanol), with continuous shaking for 30 s, following which the solution was allowed to stand for 5 min. The formation of a persistent froth indicated the presence of saponins.

Test for terpenoids/steroid (Stephen et al. 2021)

Salkowskis test

One hundred micrograms of the extract and fractions (hexane, ethylacetate, butanol) were each dissolved in 2 ml of chloroform and 3 drops of concentrated sulphuric acid (Salkowskis reagent) added at the side of the test tube. The appearance of a reddish colouration at the interface indicated the presence of terpenoids.

Liebermann–Burchard test

To 0.1 g of the extract (CME) and fractions (hexane, ethylacetate, butanol), in a test tube, was added to equal volume of acetic anhydride, and mixed together gently; 1 ml of concentrated sulphuric acid was then added to the test tube. The appearance of blue-green colour at the upper layer and a reddish, pink or purple colour at the junction of the two layers within one hour indicated the presence of triterpene.

Test for alkaloids (Stephen et al. 2021)

Dragendroff's test

One microgram of the extract and fractions (hexane, ethylacetate, butanol) were dissolved in 2 ml of 1% hydrochloric acid with vigorous stirring in a water bath. The mixture was filtered and few drops of Dragendroff's reagent added. The appearance of rose red precipitate indicated the presence of alkaloids.

Mayer's test

To 0.1 g of the extract (CME) and fractions (hexane, ethylacetate, butanol), each in a test tube, 4 drops of Mayer's reagent was added. A cream precipitate indicated the presence of alkaloids.

Test for free anthraquinones (Stephen et al. 2021)

One hundred micrograms of extract (CME) and fractions (hexane, ethylacetate, butanol), each in a test, 5 ml of chloroform was added, followed by shaking for 5 min, filtration and addition of 5 ml of 10% ammonia solution and agitation. A bright pink colour in the upper aqueous layer indicated the presence of anthraquinones.

Test for tannins

Ferric chloride test

Three drops of ferric chloride solution were added to 0.1 g each of the extract (CME) and fractions (hexane, ethylacetate, butanol), each in a test tube. Appearance of blue-black colouration indicated the presence of tannins.

Lead sub-acetate test

Four drops of lead sub-acetate solution were added to 0.1 g each of the extract and fractions in a test tube. The formation of a cream colour precipitate will indicate the presence of tannins.

Isolation, culture and recovery of infective *A. galli* eggs

Female *Ascaridia galli* were obtained from the intestine of chicken slaughtered at Sabon-Gari poultry slaughter slab in Zaria. The intestines were collected into a beaker, and transported to the Helminthology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Adult female *Ascaridia galli* worms were obtained from the infected birds according to standard parasitological techniques described by Stephen et al. (2021), Yusuf (2010) and Skallerup et al. (2005). The uteri of gravid female worms were dissected and the eggs recovered. The recovered eggs were washed with 0.5 M (KOH) solution and agitated gently for 30 min in order to dissolve the sticky albuminous layer and allow for uniform sampling. Thereafter centrifuged at $1500 \times g$ for 3 min, with supernatant decanted and eggs washed with distilled water thrice by centrifuging. The supernatant decanted and the suspended eggs were transferred into Petri dishes to which embryonating fluid (0.1 M sulphuric acid) was added before incubating for 21 days at 37°C (Das et al. 2010). The culture in test tubes was centrifuged and the eggs washed twice. The eggs were then pipetted onto the glass slide and examined under the microscope at $\times 10$ for the evidence of embryonation.

In vitro evaluation of *V. amygdalina* extract and fractions for inhibition of *A. galli* embryonation

The methods described by Gill et al. (1995) and Coles et al. (2006) were applied. Briefly, about 200 *A. galli*

eggs in 200 μ l of water were pipetted into each of 96 wells of microtitre plate. The in vitro inhibitory activity of the extract and fractions of *V. amygdalina* were evaluated at five different concentrations of 3.15, 6.25, 12.5, 25, and 50 mg/ml. Also, Albendazole was used as standard (positive) control at the same concentration of 3.15, 6.25, 12.5, 25, and 50 mg/ml. All tests were performed in triplicates. Similarly, distilled water was employed as negative control. The plate was then incubated at 37°C for 21 days (Coles et al. 1992). Thereafter, aliquots of 100 μ l from each well were pipetted onto a clean glass slide, for examination at $\times 10$ under a light microscope. The numbers of embryonated and unembryonated eggs were counted. The percent inhibition (PI) was estimated using the following formula (Coles et al., 1992):

$$PI = \frac{P_{\text{unembryo}}}{P_{\text{total}}} \times 100$$

where $P_{\text{unembryonation}}$ = Mean number of unembryonated eggs in each well. P_{total} = Mean number of eggs in each well.

Determination of median lethal dose (LD₅₀) of *V. amygdalina*

The median lethal dose (LD₅₀) of CME, EAF and BF was determined, as described by Lorke (1983). The trial was carried out in two phases using a total of twelve birds for each extract and fraction. The LD₅₀ was calculated by taking the geometric mean of the lowest dose that produced mortality and the highest dose that did not produce death was assumed to be greater than or equal to (\geq) 5000 mg/kg.

Experimental birds

A total of one hundred and fifty-day-old broiler chickens were purchased for this study, were housed and fed ad libitum (birds were fed without restriction). The birds were divided at random into 6 groups (A, B, C, D, E and F) with birds in group A, B and C further divided into 3 subgroups of ten birds each and received 200, 400 and 800 mg/kg. Birds in groups D were treated with a single dose of albendazole (200 mg/kg), while group E birds were given distilled water and served as infected non-treated controls. Birds in groups F served as the non-infected non-treated controls, respectively.

Experimental infection of birds and in vivo anthelmintic screening of *V. amygdalina* leaf extract

The embryonated eggs of *A. galli* were suspended in water inside a beaker and thoroughly agitated. Two hundred microlitre (0.2 ml) of the suspension was pipetted

and eggs in it were counted under light microscope. This was done three times and average of the eggs in it was taken. One hundred and twenty broilers were orally infected at three weeks of age with 1000 *A. galli* eggs contained in 0.5 ml of water per bird using an 18G needle attached to a 2 ml syringe. Patency was monitored by simple flotation method. Faecal drops on the wood shavings were collected from the different groups of birds starting from week three of infection and examined by the flotation method. Collection and examination of samples were done twice in a week until the infection was established using simple flotation protocol (Cringoli et al. 2010; Stephen et al. 2021). Briefly, a suspension of about 2 g of faeces in flotation fluid was filtered through layers of gauze (in a funnel) into a test tube until a convex meniscus was formed. A cover slip which had been allowed to stay on the meniscus for 3–5 min was carefully removed, placed on a clean glass slide for examination at $\times 10$ objective of a light microscope.

One hundred and twenty birds that were shedding *A. galli* in faeces were selected for the in vivo screening. The birds were divided at random into 6 groups (A, B, C, D, E and F). Groups A, B and C contain 30 birds each, while groups D and E have 10 birds each. Birds in groups A, B and C were further allocated randomly into three groups of 10 birds each (Groups A₁, A₂ and A₃; B₁, B₂ and B₃; C₁, C₂ and C₃). Groups A₁, A₂ and A₃ were treated with CME at 200, 400 and 800 mg/kg, respectively. Similarly, birds in groups B₁, B₂ and B₃ were given EAF at 200, 400 and 800 mg/kg, respectively. Also, BF at 200, 400 and 800 mg/kg was given to birds in groups C₁, C₂ and C₃, respectively. Birds in groups D were treated with albendazole (200 mg/kg), while group E birds were given distilled water (5 mg/kg) and served as infected non-treated controls. Birds in groups F served as the non-infected non-treated controls, respectively. All the treatments were given orally for three consecutive days.

Post-infection monitoring

Faecal egg count

Fresh droppings were collected from birds at day 0 (for pre-treatment FEC) and on days 7-, 14- and 21-day post-treatment from each group of bird into different sample bottles and labelled accordingly (Das et al. 2010). Faecal samples were collected from each group of birds on Egg per gram of faeces (epg) and were determined using the modified McMaster technique (Cringoli et al. 2010). Briefly, 2 g of faeces from each group was homogenized in a 28 ml flotation solution of zinc sulphate of specific gravity of 1.28. The supernatant was sieved through a tea sieve into a bottle and the sediment discarded. The supernatant from each bottle was then dispensed into both chambers of the McMaster slide using a transfer pipette,

and then allowed to stand for 5 min. The MaMaster slide was then mounted onto a microscope and viewed to count the eggs at $\times 10$ magnification. To obtain the egg per gram (epg), the total number of eggs in the two chambers was multiplied by 50, obtained thus:

$$\begin{aligned} 0.3 \text{ ml (volume of the two chambers)} &= X \text{ eggs} \\ \text{Therefore, 30 ml (final volume)} &= \frac{X}{0.3} \times 30 \text{ eggs} \\ 2 \text{ g of faeces} &= 100X \text{ eggs} \\ \text{Therefore, 1 g of faeces} &= 50X \text{ eggs} \end{aligned}$$

The anthelmintic effect of leaf extracts (CME) and fractions (BE, EAF) was determined by comparing the egg per gram (epg) of the treated group with that of those treated with distilled (untreated group) and Albendazole treated group. The percentage reduction in *A. galli* eggs in the treatment groups was calculated using the method by Kamrun et al. (2017).

$$\text{FECRT} = \frac{\text{EPG}_{(\text{pretreatment})} - \text{EPG}_{(\text{post treatment})}}{\text{EPG}_{(\text{pretreatment})}} \times 100$$

Post-mortem worm count

Three weeks post-treatment, all the birds were deprived of feed but not water for a day to empty the gastrointestinal tract for easy worm count. Three birds were randomly selected from each group and then sacrificed by euthanization. Thereafter the intestines were removed, opened and checked for the presence of adult *A. galli*. The ascarids recovered were counted and preserved in 10% buffered formalin. The percentage deparasitization was calculated (Stephen et al. 2021).

$$\frac{N - n}{N} \times 100$$

where “N” mean number of worms found in untreated control birds. “n” mean number of worms found in treated birds.

Percentage deparasitization of 70 and above was considered significant (Stephen et al. 2021; Suleiman et al. 2005).

Gross pathology

Three birds from each group were euthanized; the gastrointestinal tracts removed and observed for gross pathological lesions.

Histopathological processing and examination

Section of the small intestine was collected from each bird in all the groups for histopathological preparations and examination. The method described by Baker

et al. (2000) was used for processing for histology. The tissues were fixed in 10% buffered neutral formalin and after which they were dehydrated in ascending grades of alcohol, embedded in paraffin wax and then sectioned at 5 microns. The sections were mounted on clean grease-free slides, stained with haematoxylin and eosin (*H* and *E*) stain and were examined microscopically at different magnifications of $\times 40$, $\times 100$, $\times 200$ and $\times 400$.

Data analyses

Data obtained were expressed as mean \pm standard error of mean (SEM) and subjected to two-way analysis of variance (ANOVA) using GraphPad Prism version 8.0.2 for windows to compare the anthelmintic effect of different extract and fractions of *V. amygdalina* and presented in tables and charts. Means in different groups were compared using Bonferroni’s *post hoc* test. Values of $P \leq 0.05$ were considered significant.

Results

Feed composition

Table 1 shows the feed composition that was giving to the chickens throughout the course of the experiments.

The yield of *V. amygdalina* powdered leaves extract

Three and half kilograms (3.5 kg) of the powdered leaves of *V. amygdalina* yielded 280 g (8%) of crude methanol extract (CME). One hundred and fifty grams (53.6%) of CME was serially partition with solvents of different polarities to yield 42 g (28%) of BE, 9 g (6%) of EAF (Table 2).

Phytochemical constituents of *V. amygdalina*

The CME contained alkaloids, flavonoids, saponins, tannins, steroids, anthraquinone, cardiac glycosides and carbohydrates. The aqueous extract contained all but 3 (flavonoids, steroids and cardiac glycosides) of those

Table 1 Composition of the broiler chicken diets used for the study (CHIKUN FEED®)

Feed composition	Super starter	Finisher
<i>Ingredients (%)</i>		
Crude protein	22.0	18.0
Crude fat	4.0	5.0
Crude fiber	5.0	5.0
Calcium	1.0	0.9
Available phosphorus	0.47	0.4
Lysine	1.15	0.9
Methionine	0.5	0.4
Metabolizable energy (kCal/kg)	2900	3000

Table 2 Yield and percentage yield extract and fractions of powdered leaf of *Vernonia amygdalina* after extraction and partitioning

Substance	Quantity subjected to extraction/solvent	Resultant extracts and fractions	Yield (g)	% Yield
Powdered leaf	3.5 kg/methanol	CME	280.0	8.00
AMF	150 g/Butanol	BF	42.0	28.00
AMF	150/Ethylacetate	EAF	9.0	6.00

CME, crude methanol extracts; EAF, ethyl acetate fraction; BF, butanol fraction; HF, hexane fraction; AMF, aqueous methanol fraction

Table 3 Phytochemical constituents of extract and fractions of *Vernonia amygdalina* leaves

Constituents	CME	Aqueous methanol fraction	EAF	BF
Alkaloids	+	+	+	+
Flavonoids	+	-	+	-
Saponins	+	+	-	+
Tannins	+	+	+	+
Steroids	+	-	+	+
Cardiac glycosides	+	-	-	+
Anthraquinones	+	+	+	+
Carbohydrates	+	+	-	+

-, absent; +, present; CME, crude methanol extracts; AMF, aqueous methanol fraction; HF, hexane fraction; EAF, ethyl acetate fraction; BF, butanol fraction

found in CME, while EAF lacked saponin, cardiac glycoside and carbohydrate. The BF contained all but 1 (flavonoids) of the constituents found in the other extracts (Table 3).

Acute toxicity studies

No signs of toxicity or death were observed following individual oral administration of crude methanol extract, ethylacetate and butanol fractions of *V. amygdalina* at

doses between 10 and 5000 mg/kg. The LD₅₀ was therefore assumed to be ≥ 5000 mg/kg.

Inhibition of embryonation of *A. galli* eggs in vitro by extract and fractions of *V. amygdalina*

The results of inhibition of embryonation of *A. galli* eggs in vitro by extract and fractions of *V. amygdalina* are presented (Table 4). The mean inhibition of embryonation was significantly ($P < 0.05$) lower in the distilled water group (46.5 ± 1.5) compared to the group treated with albendazole (200 mg/ml), CME, EAF and BF fractions of *V. amygdalina*. There were significant ($P < 0.05$) differences in the inhibition of embryonation with increasing concentrations of the albendazole, extract and fractions. At 3.25 mg/ml concentration, there was no significant difference in the inhibition caused by EAF (125.5 ± 2.5) and BF (128.5 ± 0.5) and the inhibition caused by ALB (141 ± 2.0) was significantly higher compared to all the fractions and extract. Similar patterns were observed for the inhibition of embryonation at concentrations of 6.5 mg/ml, 12.5 mg/ml and 25 mg/ml. At concentration of 50 mg/ml, there was no statistically significant differences in the inhibition of embryonation by CME (189.0 ± 2.0) and ALB (194.5 ± 2.5), but these were significantly ($P < 0.05$) higher compared to those caused by EAF (162.5 ± 1.5), BF (160.5 ± 1.5).

In vitro percentage inhibition of embryonation of *A. galli* eggs by extract and fractions of *V. amygdalina*

The percentage inhibition of embryonation of *A. galli* eggs in vitro by extract and fractions of *V. amygdalina* are presented; the least percentage inhibition of embryonation was recorded for eggs exposed to distilled water (DW) (23.3%). Albendazole produced percentage inhibition of embryonation of 70.5%, 77.8%, 82.3%, 90.5% and 97.3% at concentrations of 3.25, 6.5, 12.5, 25 and 50 mg/kg, respectively. The CME inhibited embryonation of *A. galli* eggs by 66.5%, 72.3%, 79.0%, 84.8% and 94.5% at concentrations of 3.25, 6.5, 12.5, 25 and 50 mg/ml, respectively.

Table 4 In vitro mean inhibition of embryonation of *Ascaridia galli* eggs by extract and fractions of *Vernonia amygdalina*

Treatment	Concentrations (mg/ml)				
	3.25	6.5	12.5	25	50
CME	133.0 ± 2.0 ^b	144.5 ± 2.5 ^b	158.0 ± 3.0 ^b	169.5 ± 1.5 ^b	189.0 ± 2.0 ^b
EAF	125.5 ± 2.5 ^c	131.5 ± 0.5 ^c	139 ± 2.0 ^c	151.5 ± 2.5 ^c	162.5 ± 1.5 ^c
BF	128.5 ± 0.5 ^c	134.5 ± 1.5 ^c	140.0 ± 1.0 ^c	146.5 ± 1.5 ^c	160.5 ± 1.5 ^c
ALB	141 ± 2.0 ^a	155.5 ± 1.5 ^a	164.5 ± 0.5 ^a	181.0 ± 2.0 ^a	194.5 ± 2.5 ^a
DW	46.5 ± 1.5 ^e				

Values with different superscript alphabets in the same column differ significantly at $P < 0.05$

CME, crude methanol extracts; HF, hexane fraction; EAF, ethyl acetate fraction; BF, butanol fraction; ALB, albendazole; DW, distilled water

Table 5 In vitro percentage inhibition of embryonation of *Ascaridia galli* eggs by extract and fractions of *Vernonia amygdalina*

Treatment	Concentration (mg/ml)				
	3.25	6.5	12.5	25	50
CME	66.5	72.3	79.0	84.8	94.5
EAF	62.8	65.8	69.5	75.8	81.3
BF	64.3	67.3	70.0	73.3	80.3
ALB	70.5	77.8	82.3	90.5	97.3
DW	23.3				

CME, crude methanol extract; HF, hexane fraction; EAF, ethyl acetate fraction; BF, butanol fraction; ALB, albendazole; DW, distilled water

The fractions (EAF and BF) inhibited embryonation of *A. galli* eggs at ranges of 58% to 94.5% at concentrations between 3.25 and 50 mg/kg (Table 5).

Mean faecal egg counts of broiler chickens infected with *A. galli* and treated with extract and fractions of *V. amygdalina*

In all the birds infected with *A. galli* and treated with the different doses of extract and fractions (200,400 and 800 mg/kg) no significant differences ($P > 0.05$) in epg were observed. In infected chickens administered distilled water (DW), the change in FEC was not statistically significant ($P > 0.05$) throughout the period. At 7 days post-treatment, the decrease in FEC was significant ($P < 0.05$) in chickens administered CME (200 mg/kg), BF (200 mg/kg) and EAF (200 mg/kg). The decrease in FEC in chickens administered ALB (217 ± 117) and 800 mg/kg of CME (400 ± 153) showed no statistically significant

($P > 0.05$) difference at 7 days post-treatment. At 14 days post-treatment, no significant difference was observed in the decrease in FEC in chickens administered 200 mg/kg of CME, BF (200, 400 and 800 mg/kg) and EAF (400 and 800 mg/kg). At 21 days post-treatment, there was significant decreases in FEC in chickens administered albendazole (17 ± 17) and 800 mg/kg of CME (67 ± 33) compared to chickens administered 200 and 400 mg/kg of CME, all dosages of BF and EAF (Table 6).

Percentage faecal egg count reduction of *A. galli* in broiler chickens treated with extract and fractions of *V. amygdalina*

Albendazole, extract and fractions of *V. amygdalina* of all caused dose and time-dependent decreased in percentage faecal egg count reduction (FECR). Crude methanol extract (CME) caused the highest FECR followed by Butanol fraction (BF). The decreases in FEC caused by CME at 200, 400 and 800 mg/kg at day 7, 14 and 21 post-infection were significantly higher ($P < 0.05$) compared to that produced by BF and EAF. Similarly, BF at 200, 400 and 800 mg/kg produced significant ($P < 0.05$) decreased percentage FECR when compared with EAF. The FEC decreases of 81.4, 92.2 and 96.9% caused by CME at days 7, 14 and 21 post-treatment were significantly different from the respective percentage decreases of 89.2, 95.9 and 99.2 caused by ALB ($P \leq 0.05$) (Table 7).

Mean worm counts and percentage deparasitization of *A. galli* by extract and fractions of *V. amygdalina*

The mean count of *A. galli* was significantly ($P < 0.05$) higher in chickens administered distilled water

Table 6 Mean faecal egg counts of broiler chickens infected with *Ascaridia galli* and treated with extract and fractions of *Vernonia amygdalina*

Treatment	Dose (mg/kg)	Pre-treatment	Days post-treatment		
			7	14	21
DW	5 ml/kg	2650 ± 375	2150 ± 144 ^a	2300 ± 202 ^a	2350 ± 202 ^a
ALB		2000 ± 246	217 ± 117 ^d	83 ± 44 ^e	17 ± 17 ^f
CME	200	2300 ± 144	1100 ± 152 ^b	700 ± 57 ^c	650 ± 87 ^c
	400	2000 ± 180	783 ± 159 ^c	466 ± 44 ^d	283 ± 33 ^e
	800	2150 ± 288	400 ± 153 ^d	167 ± 83 ^e	67 ± 33 ^f
BF	200	2000 ± 230	1250 ± 126 ^b	750 ± 29 ^c	700 ± 29 ^c
	400	2150 ± 115	750 ± 87 ^c	650 ± 104 ^c	400 ± 29 ^d
	800	2500 ± 173	600 ± 126 ^c	500 ± 153 ^c	300 ± 76 ^d
EAF	200	2400 ± 173	1650 ± 304 ^b	1250 ± 29 ^b	950 ± 132 ^b
	400	2250 ± 202	1450 ± 284 ^b	850 ± 29 ^c	600 ± 29 ^c
	800	2500 ± 290	1100 ± 132 ^b	700 ± 200 ^c	400 ± 29 ^d

Values with the different superscript alphabets in the same row differ significantly at $P < 0.05$. Means preceded by different superscript letters differ significantly ($P < 0.05$) from one another: a and b (between DW and ALB, Extract and fractions), c and d (between ALB and the fractions), e and f (between the extract)

DW, distilled water; ALB, albendazole; CME, crude methanol extracts; HF, hexane fraction; BF, butanol fraction; EAF, ethyl acetate fraction

Table 7 Percentage faecal egg count reduction of broiler chickens infected with *Ascaridia galli* and treated with extract and fractions of *Vernonia amygdalina*

Treatments	Dosage (mg/kg)	Pre-treatment	Days post-treatment		
			7	14	21
DW	5 ml/kg	0.0	18.9	13.2	11.3
ALB		0.0	89.2	95.9	99.2
CME	200	0.0	52.2	69.6	71.7
	400	0.0	60.9	76.7	85.9
	800	0.0	81.4	92.2	96.9
BF	200	0.0	37.5	62.5	65.0
	400	0.0	65.1	69.8	81.4
	800	0.0	76.0	80.0	88.0
EAF	200	0.0	31.3	47.9	60.4
	400	0.0	35.6	62.2	73.3
	800	0.0	56.0	72.0	84.0

DW, distilled water; ALB, albendazole; CME, crude methanol extracts; BF, butanol fraction; EAF, ethyl acetate fraction

Table 8 Mean worm counts and percentage deparasitization of *Ascaridia galli* by extract and fractions of *Vernonia amygdalina*

Treatments	Dosage (mg/kg)	Mean ± SEM worm counts	% Deparasitization
DW	5 ml/kg	16.0 ± 1.0 ^a	0.0
ALB		1.0 ± 0.0 ^b	93.8
CME	200	3.7 ± 0.9 ^c	77.5
	400	2.0 ± 0.6 ^c	87.75
	800	1.3 ± 0.9 ^{bc}	91.86
BF	200	4.0 ± 0.6 ^c	75.51
	400	3.3 ± 0.9 ^c	79.61
	800	2.7 ± 0.9 ^c	85.73
EAF	200	4.0 ± 1.2 ^c	73.48
	400	4.0 ± 0.6 ^c	75.5
	800	3.0 ± 0.6 ^c	81.63

Values with the different superscript alphabets differ significantly at $P < 0.05$

DW, distilled water; ALB, albendazole; CME, crude methanol extracts; BF, butanol fraction; EAF, ethyl acetate fraction

(16.0 ± 1.0) compared to chickens in all other treatment groups (Table 8). The lowest mean count of (1.0 ± 0.0) recorded in the ALB-treated was not significantly different ($P < 0.05$) compared to the count of (1.3 ± 0.9) in chickens administered 800 mg/kg of CME. However, the mean worm count of the ALB-treated chickens was significantly ($P < 0.05$) different compared to the other treatment groups. No significant differences existed in the mean worm counts of chickens administered CME (200 and 400 mg/ml), BF (200, 400 and 800 mg/ml) and EAF (200, 400 and 800 mg/ml). There was significant

percentage deparasitization (>70%) of *A. galli* by extract and fractions of *V. amygdalina*, with the highest recorded in chickens administered 800 mg/ml (91.86%) and 400 mg/ml (87.75%) of CME and least in chickens administered 200 mg/ml (73.5%) of EAF.

Post-mortem examination of broiler chickens infected with *A. galli* and treated with extract and fractions of *V. amygdalina*

Figure 1A–L shows the histopathological changes in the various groups of broiler chickens used in this study. The histopathological findings revealed that in broiler chickens that were not infected and not treated, villi and mucosa glands were intact; while in broiler chickens that were infected and not treated, desquamation, necrosis of villi and mucosal gland occurred; in broiler chickens infected and treated with albendazole the villi and mucosa gland were intact. In those treated with the extract and the fractions, with increase in concentration, the necrosis of villi and mucosa gland was greatly reduced.

Discussion

The anthelmintic activities of crude methanol leaf extract and fractions of *V. amygdalina* against *A. galli* were evaluated in this study. The yield of 8% obtained from the leaves of *V. amygdalina* was lower than the 14.35% reported by Okoduwa et al. (2017) following cold maceration of *V. amygdalina* leaves collected from a local farm at Samaru, Zaria. Danquah et al. (2012) also reported a higher yield of 16.0% after extraction of *V. amygdalina* leaves using water. Similarly, yields of 21%, 19% and 16% were obtained after extraction of the leaves of *V. amygdalina* using microwave-assisted, soxhlet and cold extraction methods, respectively (Okoduwa et al. 2018). In similar studies, Sirama et al. (2015) reported yield of about 9% each when the root of *V. amygdalina* was extracted with acetone, methanol and water. Ekpo et al. (2007) obtained a yield of only 2% after extraction with ethanol. In this study, yield obtained after partitioning 150g of the CME was 28% (BF) which was the highest followed by EAF (6.00%). However, Okoduwa et al. (2017) reported lower yields by BF (2.39%) and EAF (1.73%) in a similar study.

The phytochemical constituents present in *V. amygdalina* leaves in this study included alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides, anthraquinones and carbohydrates. Report from other studies demonstrated the presence of these constituents in the extract and fractions of *V. amygdalina* leaves (Ekam et al. 2013; Zakaria et al. 2016; Ejiofor et al. 2017; Alara et al. 2019). In a similar study by Okoduwa et al. (2017), anthraquinones were not detected, while polyphenols

and triterpenes were present in the methanol leaf extract and fractions of *V. amygdalina*. Udochukwu et al. (2015) in addition, the presence of oxalate, phytate, phenols and cyanogenic glycosides in the aqueous and ethanolic leaf extracts of *V. amygdalina*. Also, the presence of phenolics and phlobatannins has been reported in the root and stem bark extracts of *V. amygdalina* collected from Lapai, Niger State (Audu et al. 2018). The differences in the yield of extracts and fractions phytochemical constituents could have resulted from differences in extraction method used, drying method, type and volume of solvent, season in which plant was obtained, part of plant used, soil characteristics, environmental factors and geographical locations (Ncube et al. 2012). The LD₅₀ of the extract and fractions *V. amygdalina* tested individually was ≥ 5000 mg/kg. Similarly, there were no signs of toxicity or death in following oral administration of the crude methanol extract, ethylacetate and butanol fractions. This was higher than the LD₅₀ of 1950 mg/kg reported by Sha'a et al. (2011) in rats and 2500.62 ± 5.24 mg/kg by Mebratu et al. (2013) in mice. Also, in a study by Ekpo et al. (2007), neither signs of toxicity nor mortality were observed in rats administered ethanolic leaf extract of *V. amygdalina* at 100–1000 mg/kg. Hence, the increased LD₅₀ could probably be due to increased tolerance to the extract resulting from the larger size and weight of the chickens.

The methanol leaf extract and fractions of *V. amygdalina* demonstrated anthelmintic activities in vitro and in vivo against *A. galli* in this study as evidenced by inhibition of embryonation, faecal egg count reduction, decreased mean worm counts and increased percentage deparasitization. The patterns of these anthelmintic activities were also observed to be concentration-dependent as evidenced by increased activities with increase in concentration of the extract and fractions in our study. At

highest concentration (50 mg/ml) and dosage of (800 mg/kg), CME produced the highest activities in vitro next to albendazole with no significant difference when compared to albendazole. Islam et al. (2008) reported that the inhibition of embryonation of *A. galli* eggs was increased with increase in concentrations of ethanolic leaf extract of some indigenous plants in Bangladesh. Also, the increase in concentrations of aqueous and ethanol leaf extracts of *V. amygdalina* resulted in increased mortality of *Heligmosomoides bakeri* (Nweze et al. 2013) and increased inhibition of motility of *Ascaris suum* (Nalule et al. 2013) in vitro. Safitri et al. (2019) also reported that increasing concentration of *V. amygdalina* caused increased anthelmintic activity against *A. galli* worms in vitro. The enhanced activity with increasing concentrations could be due to saturation of target receptors by increased active ingredients resulting in hyperpolarization of parasite membranes (Lullman et al. 1993; Nalule et al. 2013). This hyperpolarization will in turn limit excitation and impulse transmission leading to flaccid paralysis of worm muscles.

The anthelmintic activity of *V. amygdalina* against *A. galli* in vitro and in vivo in this study could be due to the presence of secondary metabolites such as alkaloids, glycosides, saponin and tannin (Nalule et al. 2013). Though these metabolites are unstable, their biological activity is dependent on their structure, physical and chemical properties, and method of extraction (Waterman 1992; Alawa et al. 2003). The increased anthelmintic activity by CME could possibly be due to the presence of large amount of these metabolites compared to the fractions. Also, the findings of previous studies suggest that alkaloids, glycosides, saponins and tannins possess concentration-dependent anthelmintic activity either singly or in synergy (Kaufman et al. 1999; Githiori et al. 2006; Wynn and Fougere 2007; Islam et al. 2008). The nematicidal

(See figure on next page.)

Fig. 1 Showing microscopic lesions in the intestine of chicken infected with *Ascaridia galli* and treated with Amygdalina Vernonia extracts.

A Photomicrograph of section of jejunum of uninfected broiler chicken showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **B** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered distilled water. Note desquamation and necrosis of villi (N) and presence of *Ascaridia galli* eggs (arrows). *H & E* $\times 250$. **C** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered albendazole showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **D** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 800 mg/kg CME *Vernonia amygdalina* showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **E** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 400 mg/kg CME *Vernonia amygdalina* showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **F** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 200 mg/kg CME *Vernonia amygdalina* showing slight desquamation of villi (arrows). *H & E* $\times 250$. **G** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 800 mg/kg BF *Vernonia amygdalina* showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **H** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 400 mg/kg BF *Vernonia amygdalina* showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **I** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 200 mg/kg BF *Vernonia amygdalina* showing slight desquamation of villi (arrows). *H & E* $\times 250$. **J** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 800 mg/kg EAF *Vernonia amygdalina* showing intact villi (V) and mucosal glands (M). *H & E* $\times 250$. **K** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 400 mg/kg EAF *Vernonia amygdalina* showing desquamation of villi (arrows). *H & E* $\times 250$. **L** Photomicrograph of section of jejunum of broiler chicken infected with *Ascaridia galli* eggs and administered 200 mg/kg EAF *Vernonia amygdalina* showing desquamation of villi (arrows). *H & E* $\times 250$

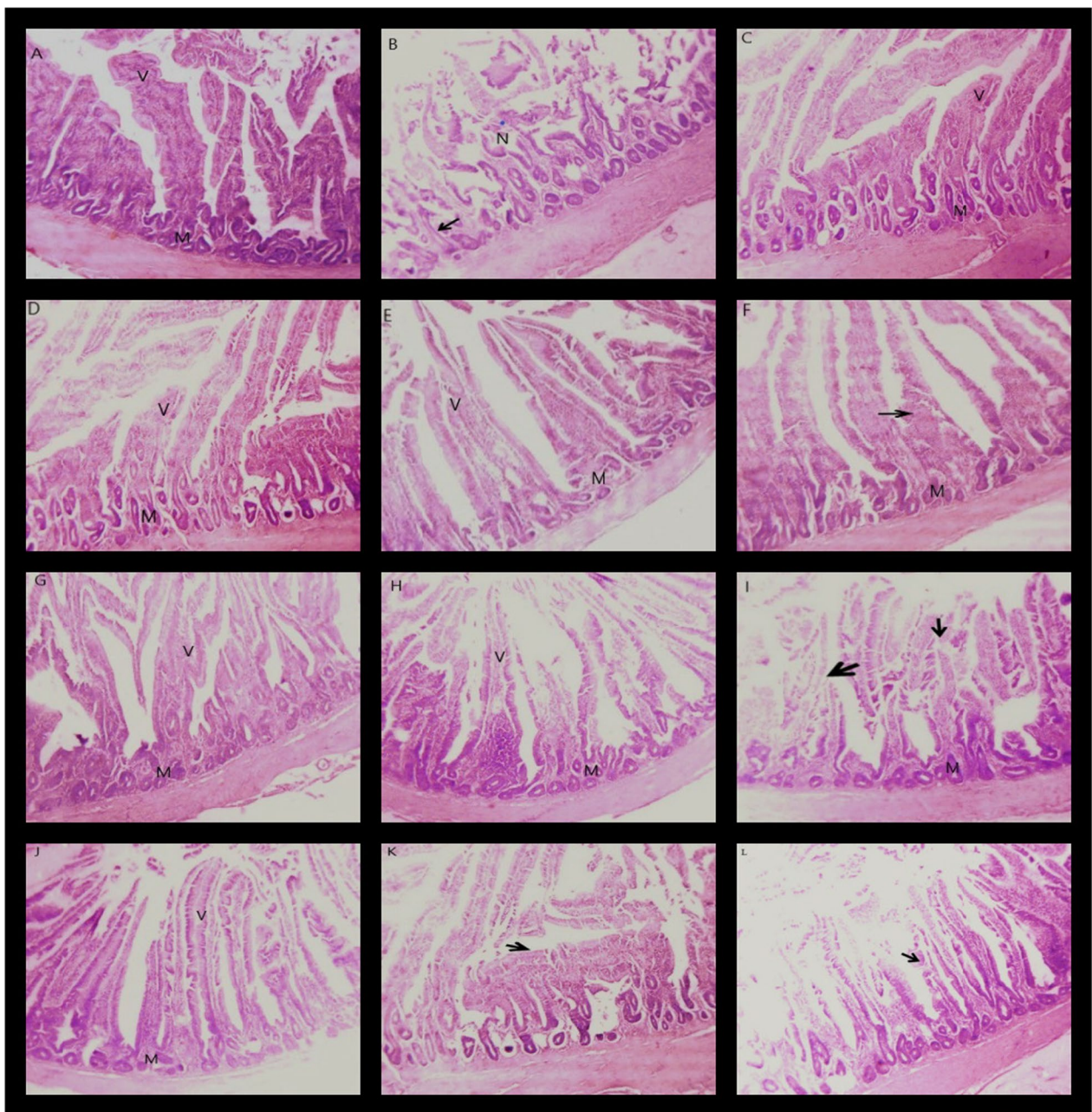


Fig. 1 (See legend on previous page.)

activity of tannins has been shown to be a result of interference with energy generation in helminths parasites by uncoupling of oxidative phosphorylation, which is similar to that of niclosamide and oxiclozanide (Martin, 1997; Ademola and Idowu 2006; Kotze et al. 2009). The disruption of motility, feed absorption and reproduction by the binding action of tannins to glycoprotein on the cuticle of the parasite could also contribute to the anthelmintic activity observed in this study (Aerts et al. 1999;

Githiori et al. 2006). On post-mortem examination, there were intact villi and mucosal glands in the jejunum of infected chickens administered 400 and 800 mg/kg CME, BF and EAF of *V. amygdalina* and desquamation of villi in chickens administered 200 mg/kg of *V. amygdalina*. The lesions in chickens administered lower dosages of the extracts and fraction could be due to pressure necrosis resulting from worm load on the villi. Adult worms have been reported to migrate up and down the intestinal

lumen when present in large numbers (Ikeme 1971) and thus, could be responsible for the desquamation of villi observed in this study.

Conclusions

From this study, extract and fractions of *Vernonia amygdalina* showed anthelmintic efficacy against *A. galli* by the inhibition of embryonation of eggs, reduction in faecal egg count and the number of the parasite and can therefore be used as an alternative to regular anthelmintic drug. However, further study should be carried out to ascertain the mechanism of action of these extracts and fractions.

Abbreviations

EVM: Ethnoveterinary medicine; ALB: Albendazole; CME: Crude methanol extract; EAF: Ethyl-acetate fraction; BF: Butanol fraction; FEC: Faecal egg count; FAO: Food and Agricultural Organization; ABU: Ahmadu Bello University; HF: Hexane fraction; ml: Mills; g: Grams; M: Mole; KOH: Potassium hydroxide; *g*: Gravity; PI: Percentage inhibition; P: Percentage; LD₅₀: Lethal dose; Mg: Milligrams; Kg: Kilograms; egg: Egg per grams; FECRT: Faecal egg count reduction test; SEM: Standard error of mean; ANOVA: Analysis of variance; DW: Distilled water; FECR: Faecal egg count reduction.

Acknowledgements

The authors wish to acknowledge assistance kindly rendered by the laboratory staff of the Department of Veterinary Pharmacology and Toxicology, Department of Veterinary Parasitology and Entomology and Pharmaceutical Science, all of Ahmadu Bello University Zaria.

Author contributions

KAG, OJA, MMS and SD designed the experiment and contributed in the discussion along with KHY, OO read and interpreted the histopathological slides while SK and MO contributed in the statistical analysis. All authors read and approved the final manuscript.

Funding

The authors declare that no funding was received support from any organization/institution or personality for the purpose of this study or in preparation of manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for the use of animals for this study was approved by the Ahmadu Bello University, Committee on Animal Use and Care (ABU-CAUC/2021/042), A.B.U Zaria; all authors gave consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria. ²Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. ³Department of Pathology,

Ahmadu Bello University, Zaria, Nigeria. ⁴Department of Zoology, Ahmadu Bello University, Zaria, Nigeria.

Received: 17 March 2022 Accepted: 23 April 2022

Published online: 08 May 2022

References

- Abdalla AH, Abeer EM, Rasha AHA, Enas AMH (2012) Evaluation of the ethanolic extracts of three plants for their molluscicidal activities against snail's intermediate hosts of *Schistosoma mansoni* and *Fasciola*. *Int J Basic Appl Sci* 1(3):235–249
- Ademola IO, Idowu SO (2006) Anthelmintic activity of *Leucaena leucocephala* seed extract on *Haemonchus contortus*-infective larvae. *Vet Rec* 158:485–486
- Aerts RJ, Barry TN, McNabb WC (1999) Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agric Ecosyst Environ* 75:1–12
- Ajala MK, Nwagu BI, Otchere EO (2007) Socioeconomics of free-range poultry production among Agropastoral women in Giwa Local Government Area of Kaduna State, Nigeria. *Niger Vet J* 28:11–18
- Alara OR, Abdurahman NH, Abdul Mudalip SK (2017) Phytochemical and pharmacological properties of *Vernonia amygdalina*: a review. *J Chem Engine Indian Biotech* 2:80–96
- Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA (2019) Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. *J Taibah Univ Sci* 13(1):414–422
- Alawa CBI, Adamu AM, Gefu JO, Ajanusi OJ, Abdu PA, Chiezey NP, Alawa JN, Bowman DD (2003) In vitro screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. *Vet Parasitol* 113:73–81
- Audu I, Shuaibu BS, Alona CL, Murtala MM, Jiya JA (2018) Phytochemical analysis and antimicrobial activity of bitter leaf (*Vernonia amygdalina*) collected from Lapai, Niger State, Nigeria on some selected pathogenic microorganisms. *Sci World J* 13(3):15–18
- Baker J, Silverton RE, Pillister CJ (2000) Introduction to medical laboratory technology: dehydration, impregnation, embedding techniques and section preparation, 7th edn. Hodder Education Publishers, London, pp 199–242
- Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ (1992) World association for advancement of veterinary parasitology: method for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 44:35–44
- Coles GC, Stafford KA, Mackay PH (1998) Ivermectin-resistance *Cooperia* species from calves on a farm in Somerset. *Vet Rec* 142:255–256
- Coles GC, Jackson F, Pomroy WF, Prichard RK, Samsom-Himmelstjerna G, Silvester A, Taylor MA, Vercruyse J (2006) The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 136:167–185
- Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and human. *Nat Protoc* 5(3):503–515
- Danquah CA, Koffuor GA, Annan K, Ketor EC (2012) The anthelmintic activity of *Vernonia amygdalina* (Asteraceae) and *Alstonia boonei* de wild (Apocynaceae). *J Med Biomed Sci* 1(1):21–27
- Das G, Kaufmann F, Abel H, Gauly M (2010) Effect of extra dietary lysine in *Ascaridia galli*-infected grower layers. *Vet Parasitol* 170:238–243
- Douglas EC, Nelson AC, Mark A, Harrison JL, Wilson RJB, Paula JF (2015) *Salmonella* and antimicrobial resistance in broilers: a review. *J Appl Poult Res* 24(3):408–426
- Ejiofor IL, Zaman K, Das A (2017) Effect of extracts of *Vernonia amygdalina* in helminthiasis: a tropical neglected disease. *Open Access J Pharmaceut Res* 1(8):1–6
- Ekam VS, Ebong PE, Johnson JT, Dasofunjo K (2013) Effect of activity directed fractions of *Vernonia* on total body weight and blood glucose levels of diabetic Wistar albino rats. *Int J Adv Sci Technol* 2:153–157
- Ekpo A, Eseyin OA, Ikpeme AO, Edoho EJ (2007) Studies on some biochemical effects of *Vernonia amygdalina* in rats. *Asian J Biochem* 2:193–197
- Fakae BB, Paul-Abiade CU (2003) Rainy season period prevalence of helminthes in the domestic fowl (*Gallus gallus*) in Nsukka, Eastern Nigeria. *Niger Vet J* 24:21–27

- Farombi EO, Owoeye O (2011) Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *Int J Environ Res Public Health* 8:2533–2555
- Food and Agricultural Organization (2010) Poultry meat and eggs: agribusiness handbook. Director of Investment Centre Division, FAO, Rome, p 77
- Gill JH, Redwin JM, Van Wyk JA, Lacey E (1995) Avermectin inhibition of larval development in *Haemonchus contortus*: effects of ivermectin resistance in nematodes. *Vet Parasitol* 25:463–470
- Githiori JB, Athanasiadou SY, Hamsborg SM (2006) Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet Parasitol* 139:303–320
- Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008) Extraction technologies for medical and aromatic plants. *Int Centre Sci High Tech* 3:22–25
- Idika IK, Obi CF, Ezeh IO, Njoku IN, Nwosu CO (2016) Gastrointestinal helminth parasites of local chickens from selected communities in Nsukka region of South Eastern Nigeria. *J Parasitol Dis* 40(4):1376–1380
- Ikeme MM (1971) Observations on the pathogenicity and pathology of *Ascaridia galli*. *Parasitology* 63(2):169–179
- Islam KR, Farjana T, Begum N, Mondal MMH (2008) *In vitro* efficacy of some indigenous plants on the inhibition of development of eggs of *Ascaridia galli* (Digenia: Nematoda). *Bangladesh J Vet Med* 6(2):159–167
- Kamrun N, Md Kamal H, Kaiser H, Tony G (2017) Development, efficacy and toxicity study of novel moxidectin loaded freeze-dried seed mix for the management of internal parasite in caged and aviary birds. *J Pharmacol Sci Res* 9(9):1453–1462
- Kaufman PB, Cseke LJ, Warber S, Duke JA, Briellmann HL (1999) Natural products from plants. CRC Press, Boca Raton, pp 12–43
- Kotze AC, O'Grady J, Emms J, Toovey AF, Hughes S, Jessop P, Bennell M, Vercoe PE, Revell DK (2009) Exploring the anthelmintic properties of Australian native shrubs with respect to their potential role in livestock grazing systems. *Parasitology* 136(9):1065–1080
- Lawal JR, Jajere SM, Geidam YA, Bello AM, Wakil Y, Mustapha M (2015) Antibiotic residues in edible poultry tissues and products in Nigeria: a potential public health hazard. *Int J Ani Vet Adv* 7(3):55–61
- Lorke D (1983) A new approach to practical acute toxicity testing. *Arch Toxicol* 54:257–287
- Lullman HK, Morh M, Bieger D (1993) Colour atlas of pharmacology. Theme Medical Publisher Inc., New York, pp 52–98
- Martin RJ (1997) Modes of action of anthelmintic drugs. *Vet J* 154(1):11–34
- Mathius-Mundy M (2004) Ethnoveterinary medicine: harnessing its potential. *Vet Bull* 74(8):27N–37N
- Mebratu A, Yamrot K, Eyasu M, Yonas B, Kelbesa U (2013) Toxic effects of aqueous leaf extract of *Vernonia* *abyssinica* on blood, liver and kidney tissues of mice. *Momona Ethiop J Sci* 5(2):15–31
- Nalule AS, Mbaria JM, Kimenju JW (2013) *In vitro* anthelmintic potential of *Vernonia amygdalina* and *Secamone africana* on gastrointestinal nematodes. *Agric Biol J N Am* 4(1):54–66
- Naphade ST (2014) A survey on prevalence of helminth infection in Desi poultry birds from Marthawada region of Maharashtra. *India Int J Res Biosci* 3(1):13–18
- Ncube B, Van Staden FJF (2012) Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. *S Afr J Bot* 82:11–20
- Newbold LK, Burthe SJ, Oliver AE, Gweon HS, Barnes CJ, Daunt F, Van der Gast CJ (2017) Helminth burden and ecological factors associated with alterations in wild host gastrointestinal microbiota. *ISME J* 11:663–675
- Nweze NE, Ogidi A, Ngongeh LA (2013) Anthelmintic potential of three plants used in Nigerian ethnoveterinary medicine. *Pharm Biol* 51(3):311–315
- Ogbaje CI, Agbo EO, Ajanusi OJ (2012) Prevalence of *Ascaridia galli*, *Heterakis gallinarum* and tapeworm infection, in birds slaughtered in Makurdi Township. *Int J Poult Sci* 11(2):103–107
- Okoduwa SIR, Umar IA, James DB, Inuwa HM (2017) Validation of the anti-diabetic effects of *Vernonia amygdalina* Delile leaf fractions in fortified diet-fed streptozotocin-treated rat model of type-2 diabetes. *J Diabetol* 8(3):74–85
- Okoduwa SIR, Okpe O, Okoduwa UJ, Igiri BE, Mhya DH, Mbora LO (2018) Comparison of yield and phytoconstituents of *Vernonia amygdalina* and *Ocimum gratissimum* leaves extract from three extraction methods. *Int J Biomed Clin Sci* 3(2):27–34
- Ola-Fadunsin SD, Abdullah D, Hussain K, Rabiu M, Ganiyu IA, Karaye PG (2019) Review on avian helminthoses diagnosed in Osun state veterinary clinics, southwest, Nigeria. *Ife J Sci* 21:1. <https://doi.org/10.4314/ijfs.v21i1.10>
- Safitri AD, Hamid IS, Hastutiek P, Koesdarto S, Sugihartuti R, Suprihati E (2019) The anthelmintic activity of ethanol extract of bitter leaf (*Vernonia amygdalina*) against *Ascaridia galli* worm in vitro. *J Parasit Sci* 3(1):19–22
- Sahu S, Sinha KP (2016) Studies on the prevalence of helminthic infection in Desi poultry birds from Darbhanga region of North Bihar, India. *Int J Fauna Biol* 3(3):87–90
- Shaa KK, Oguche S, Watila IM, Ikpa TF (2011) *In vitro* antimalarial activity of the extracts of *Vernonia amygdalina* commonly used in traditional medicine in Nigeria. *Sci World J* 6(2):5–9
- Silva GS, da Romera DM, Fonseca LEC, Meireles MV (2015) Helminthic parasites of chickens (*Gallus domesticus*) in different regions of São Paulo State. *Brazil Rev Bras Cienc Avic* 18(1):163–168
- Sirama V, Kokwaro J, Owuor B, Yusuf A, Kodhiambo M (2015) *In vitro* anthelmintic activity of *Vernonia amygdalina* Del. (*Asteraceae*) roots using adult *Haemonchus contortus* worms. *Int J Pharm Res* 5(1):1–7
- Skallerup P, Luna LA, Johansen MV (2005) The impact of natural helminthic infections and supplemental protein on growth performance of free-ranging chickens on small holder farms in El Sauce, Nicaragua. *J Prev Vet Med* 69(3–4):229–244
- Stephen K, Ajanusi OJ, Suleiman MM, Orakpoghenor O, Ogwiji M (2021) *In vitro* and *in vivo* anthelmintic effects of *Stereospermum kunthianum* (Cham-holl) leaf extracts against *Ascaridia galli* in experimentally infected broiler chickens. *J Parasit Dis*. <https://doi.org/10.1007/s12639-021-01426-6>
- Suleiman MM, Mamman M, Aliyu YO, Ajanusi JO (2005) Anthelmintic activity of the crude methanol extract of *Xylopiya aethiopicum* against *Nippostrongylus brasiliensis* in rats. *Vet Arch* 75:487–495
- Treas and Evans (1996) Trease and evans pharmacognosy, 12th edn. Billiere Tindall Edinborough, London, pp 546–547
- Udochukwu U, Omeje FI, Uloma IS, Oseiwe FD (2015) Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *Am J Res Commun* 3(5):1–11
- Waterman PG (1992) Role for secondary metabolites in plants. *Ciba Found Sympos* 171:255–275
- Wynn SG, Fougere BJ (2007) Introduction: why use herbal medicine. In: Wynn SG, Fougere BJ (eds) *Veterinary herbal medicine*. Library of congress cataloging-in publication data, pp 695–696. ISBN: 10:0-323-029981
- Yousfi F, Senouci K, Medjoul I, Djellil H, Slimane TH (2013) Gastrointestinal helminths in the local chicken *Gallus gallus domesticus* (Linnaeus) in traditional breeding of North Western Algeria. *Biodivers J* 4(1):229–234
- Yusuf KH (2010) *Ascaridia galli* in birds: prevalence and comparative effect of oral and parental Ivermectin in two breeds of broilers (unpublished)
- Zakaria Y, Azlan NZ, Fakhruddin N, Hassan N, Muhammad H (2016) Phytochemicals and acute oral toxicity studies of the aqueous extract of *Vernonia amygdalina* from state of Malaysia. *J Med Plants Stud* 4:1–5

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.