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# Evaluation of Anthelmintic Activities of Fractions of *Acanthus Montanus* (Acanthaceae) on Adult *Heligmosomoides Bakeri* (Nematoda, Heligmosomatidae)

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## Abstract

*Acanthus montanus* Nees T. Anderson (Acanthaceae) has been employed in folk medicine for treatment of different kinds of ailment, but there is dearth of documented information on its therapeutic activities against parasites. In this study, pulverized *Acanthus montanus* leaf was subjected to four different extraction techniques. The percentage of yields were 25.58%, 31.42%, 11.58% and 3.00% weight by weight *(w/w)* of crude ethanol extract (CEE), aqueous (AQ), *n*-butanol (BUT) and chloroform (CHLO) portions, respectively. All the extracts, excluding the chloroform portion were administered to worm-infested mice *per os* at dose of 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg each for five days consecutive. Mice were euthanized and the adult worm counted for rates of deparasitization. The aqueous extract did not cause significant deparasitization even at the highest dose of 2.0 g/kg. The CEE caused significant ($p<0.05$) deparasitization rate of 72.35% at 2.0 g/kg dose. The *n*-butanol portion caused significant ($p<0.05$) deparasitization rates at doses between 1.4 mg/kg and 2.0 mg/kg (86.17% and 97.04% respectively) compared to figures from distilled water-treated mice (Control) as well as those from mice treated with the aqueous or crude ethanol portion. The 97.04% deparasitization produced by the 2.0 g/kg dose was not stastistically different ($p>0.05$) from the 100% deparasitization obtained using albendazole at the manufacturer's recommended dose of 10 mg/kg. This study has demonstrated that the *n*-butanol extract of *Acanthus montanus* leaf has profound anthelmintic activity against experimental *Heligmosomoides bakeri* infection in mice. Further phytochemical analysis and evaluation is being advocated in large animals and possibly human subjects.

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## Introduction

Helminths are a major cause of reduced productivity in livestock in many countries, particularly the tropics [1]. Among the three main classes of helminths (Cestoda, Trematoda and Nematoda) that exist, the Class Nematoda contains the most pathogenic helminths of livestock and companion animals, hence a threat to successful and sustainable livestock production worldwide [2]. These helminth infections cause direct and indirect losses. Direct losses are due to drop in production and death of animals, while indirect losses are due to cost incurred on control strategies such as cost of drugs, labour and drenching equipment. Other helminth-related setbacks include delay in achieving target weights, reduced quality of carcass and predisposition to other diseases [3].

*Heligmosomoides bakeri,* a parasite of laboratory mouse, *Mus musculus* [4] of the Subfamily Heligmosominae, closely related to species of economic importance contained in the Subfamily Trichostrongylinae [5] is the ideal model for experimental works. This nematode is widely used as a gastrointestinal parasitic model in immunological, pharmacological, and toxicological studies [6]. Many anthelmintic studies have been carried out using *H. bakeri* [7-9]. It is a convenient parasite to conduct experimental work because of its short and direct life cycle [10].

Systemic anthelmintics have long been considered the most effective way of controlling helminth infections, to minimize losses. However, the threats of anthelmintic resistance and risk of residue in meat and milk are of concern. The availability and affordability of systemic anthelmintics to small holder farmers and pastoralists is a major problem in many developing countries. These setbacks justify the need for the use of traditional medicine plants [11] in different parts of the world. The screening and proper evaluation of medicinal plants could offer possible alternatives that may both be sustainable and environmentally acceptable [12].

*Acanthus montanus* (Nees) T. Anderson, a member of the Acanthaceae family [13] have been used to treat various ailments in Africa. This prickly perennial shrub variously known as "Bear's breech", "Mountain thistle", "Alligator plant" and "Thorny pigweed" [13,14] has been employed in folk medicine by the Igede people of Benue State, Nigeria for treatment of different kinds of ailments [15], and positive results seem to be associated with the folkloric use of the leaves of this plant, *"Idumngbe"*, as called by the Etulo natives in Benue State, Nigeria, in the treatment and control of gastrointestinal worms in children and adults [16]. [17] reported that *A. montanus* might have anthelmintic property. Although some studies [16,17] have been conducted on the anthelmintic properties of *A. montanus* leaf extract, no study has established the anthelmintic properties of different fractions of this plant on *H. bakeri.* In the present study, *in vivo* study was performed to evaluate the anthelmintic properties of aqueous, crude ethanol and *n*-butanol extracts of the leaves of *A. montanus* on the adult *H. bakeri*, the trichostrongyloid model of the laboratory mouse [4], with the view to determining the minimum concentration that has significant anthelmintic activity.

## Materials and Methods

### *Plant Material Collection and Identification*

Fresh leaves of *A. montanus* with stalks were collected/ harvested in the months of March and April along a stream in northern part of Katsina-Ala township of Katsina-Ala Local Government Area of Benue State. Katsina-Ala town is located on latitude 7º 10′ N and longitude 9º 19′ E in the middle belt

(Guinea Savannah) of Nigeria. A sample of the plant was brought to Zaria and was identified/ authenticated by a plant taxonomist at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher specimen was deposited and assigned a voucher number 7037.

*Preparation and Preservation of Extracts*

The harvested leaves were air-dried at room temperature until a constant weight was obtained, then pounded in a wooden mortar with pestle. The pulverized product weighing about 6 kg was stored in an air-tight nylon bag under cool, dark conditions at room temperature until use.

During extraction, 1 kg (250 g per separating funnel) of the pulverized product was soaked in 95% ethanol (EtOH) in the ratio 1:6 *w/v* in four separating funnels (pre-plugged with cotton wool) for 72 hours. The extracted solution collected in excess of the solvent was transferred in evaporating dishes and concentrated to dryness over water baths at 60ºC. The dried extract obtained after concentration was then weighed to determine the percentage yield. Thereafter, 100 g of the mixture was suspended in 300 mL of 17.65% methanol (MeOH) in a large beaker. The solution was then partitioned with chloroform in equal volume in a separating funnel to yield chloroform (CHLO) and aqueous (AQ) portions. Lastly, the aqueous portion was further partitioned with *n*-butanol in equal volume also, to obtain final *n*-butanol (BuOH/BUT) and aqueous extracts. 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 again concentrated to residue over the water bath at 60ºC and weighed to determine percentage yield in terms of the mass of the crude ethanol extract (CEE). These different fractions of leaf extracts of *A. montanus* were packed in clean air-tight glass bottles and stored at room temperature in the laboratory hood until used.

*Qualitative Phytochemical Screening of Crude Ethanolic Leaf Extracts of A. Montanus*

One gramme of crude ethanol leaf extracts of *A. montanus* was dissolved in 100 mL of distilled water in three test tubes and subjected to qualitative phytochemical screening employing standard screening tests [18].

*Thin Layer Chromatography*

Thin Layer Chromatography (TLC) was carried out on the crude ethanol leaf extracts of *A. montanus* and the different fractions thereof using the method of Mehta [19]. Little quantities of the Crude Ethanol, *n*-Butanol, Chloroform and Aqueous Extract of *A. montanus* leaf were fetched with a spatula and dissolved in 5 mL in their respective solvents in well labeled Bijou bottles. These were used to spot 10 cm x 5 cm TLC plates (already coated with 0.25 mm silica gel 60F254 that were marked at about 1.0 cm intervals and 1.5 cm from the bottom) using capillary tubes and were allowed to air dry. The plates were then placed (the spotted portion downwards) in an air-tight TLC tank that was already charged with eluting solvent (chloroform-ethyl acetate-formic acid, 5:4:1). The respective plant constituent(s) were allowed to drag upward along the stationary phase from the spots by capillary action until the solvent front was seen reaching the top. The plates were then removed, marked, air-dried and sprayed with the detecting (spray) reagents (1% $FeCl_3$, Dragendoff's and Anisaldehyde-$H_2SO_4$ in 10% ethanol) [20] in the fume hood to develop the chromograms. Retention factor *(Rf)* value of the compound(s) was calculated by dividing the distance travelled by the compound (solute) by that travelled by the solvent front [18,19].

*Experimental Animals Used in Anthelmintic Studies*

Ninety (5-10 weeks) apparently healthy albino mice, *Mus musculus* of both sexes weighing between 15 and 25 g were obtained and were housed in well fenestrated plastic cages and allowed free access to standard feed and tap water. Within the two weeks of acclimatization, faecal pellets obtained *per rectum* from the group set aside for anthelmintic studies were analysed and the mice treated with broad spectrum anthelmintic, albendazole at 10 mg/kg for deparasitization.

*The Parasite Heligmosomoides Bakeri*

*Heligmosomoides bakeri* adult were obtained from Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka-Nigeria where they were maintained in mice. Both infected mice and infective ($L_3$) larvae were transported to Ahmadu Bello University, Zaria. They

were immediately multiplied and maintained under laboratory conditions.

*Faecal Sample Collection, Coproculture and Recovery of Infective $L_3$ of H. Bakeri*

The modified method of [21] was used to obtain clean infective larvae used for anthelmintic trial. The larval suspension was allowed to stand on the bench for one hour. The supernatant fluid was carefully aspirated using Pasteur pipette. To ensure an even distribution of the larvae, the suspension was mixed by gentle shaking of the tube. Thereafter, an aliquot of 0.1 mL was drawn onto a clean microscope slide and larvae counted under x4 objective. This was repeated four more times, after which mean count obtained was used to determine the number of larvae/mL of the suspension.

*Experimental Infection of Mice with H. Bakeri $L_3$*

Experimental infection of mice was by oral route (gavage). Mice were well restrained at the scruff and 0.1 mL of the larval suspension inoculated right into the oesophagus via a blunted tip slightly curved 18 gauge needle mounted on tuberculin (1 mL) syringe. One hundred and fifty (150) infective larvae ($L_3$) of *H. bakeri* were inoculated into each worm-free mouse.

*Treatment Groups*

Seventy (70) mice positive for ova of *H. bakeri* at 15$^{th}$ day post infection (PI) were randomly assigned into 14 experimental (treatment) groups of five mice each. Twelve (12) groups were administered with four (4) graded doses of CEE, BUT and AQ fractions. The remaining two (2) groups were controls, {positive control (Albendazole, ABZ 10 mg/kg) and negative control (Distilled water, DW 5 mL/kg)}. Each of the estimated doses of the various fractions of *A. montanus* were administered to mice (according to grouping previously described) for five consecutive days; on the 18$^{th}$ to 22$^{nd}$ days post infection (PI) [22]. Doses were chosen at a common logarithmic interval of 0.08 for all extract-treated groups. All treatments were administered orally.

*Postmortem Worm Count*

On the 23$^{rd}$ day PI, all the mice were deprived of food but not water for 24 hours so as to empty the gastrointestinal tract to make worm counting easier. Mice were euthanized in chloroform chamber and necropsied. Modified method [23] was used for worm count.

*Anthelmintic Evaluation*

Anthelmintic efficacies of the extracts were accessed by counting the worms in the treated animals and comparing with counts from the untreated control mice. The percentage deparasitization (DP), (%DP- reduction in the worm counts) for the various groups was then calculated using the formula:

$$\%DP = \frac{N-n}{N} \times 100$$

Where: N= mean worm count in untreated group.

n = mean worm count in treated groups [24].

Seventy percent (70%) reduction in worm count or more was considered significant at $p<0.05$ [8].

*Data Analysis*

The worm count data generated were summarized in tables and expressed in percentages (percentage deparasitization). One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Post Test (Graphpad Instat) were used for data analysis. The results were expressed as Mean ± SEM. Difference between Means of treated and untreated control groups was considered significant at $p<0.05$.

**Results**

Extraction of pulverized leaf of *A. montanus* showed that 1,000 g yielded 255.84 g of crude ethanol (EtOH) extract. Solvent partitioning of 100 g of crude (EtOH) extract yielded 31.42 g, 11.58 g and 3.00 g of aqueous (AQ), *n*-butanol (BuOH) and chloroform (CF) fractions (extracts) respectively. The remaining portion was discarded as residue. The colour and percentage yields of the plant material is as shown in Table 1. Generally, partitioning with water resulted in the highest quantity of crude extract, while CF gave the least quantity. Figure 1

*Qualitative Phytochemical Screening of Crude Ethanolic Leaf Extracts of A. Montanus*

The results of qualitative phytochemical screening of CEE of *A. montanus* are as shown in Table 2. The major phytochemicals present in *A. montanus* leaf extract include glycosides, unsaturated steroids and triterpenes, saponins, tannins, flavonoids and alkaloids.

*Thin Layer Chromatography (TLC)*

Table 1. Extract yield (% dry weight) from *A. montanus* leaf.

| Extract | Colour | Yield *(% w/w)* |
|---|---|---|
| Crude EtOH Extract (CEE) | Brown | 25.58 |
| Aqueous (AQ-extract) Fraction | Light-brown | 31.42 |
| BuOH Fraction (*n*-butanol extract) | Dark-brown | 11.58 |
| Chloroform Fraction (CF-extract) | Dark-green | 3.00 |

Table 2. Results of qualitative phytochemical screening of CEE of *A. montanus.*

| Constituent(s) | Test | Observation | Inference |
|---|---|---|---|
| Carbohydrates | Molish | Violet ring colour | + |
| Glycosides | Ferric chloride | Dark brown coloration | + |
| Free Anthracene Derivative | Bontrager's | Light yellow coloration | – |
| Combined Anthracene Derivative | Modified Bontrager's | Light yellow coloration | – |
| Unsaturated Steroid | Liebermann Burchard | Yellowish brown ppt | + |
| Triterpenes | Liebermann Burchard | Reddish coloration | + |
| Unsaturated sterols | Salkowski | Brownish coloration | + |
| Cardiac glycoside | Keller-kiliani | Purple brown colora-tion | + |
| Saponin glycoside | Frothing | Persistent (honey comb) froth | + |
| Tannins (condensed) | Ferric chloride | Greenish-black ppt | + |
| Tannins (hydrolysable) | Ferric chloride | Greenish-black ppt | – |
| Flavonoids | Shinoda | Dark red coloration | + |
| Flavonoids | Sodium hydroxide | Yellow coloration | + |
| Alkaloids | Mayer's | Creamy white ppt | + |
| Alkaloids | Wagner's | Reddish brown ppt | + |
| Alkaloids | Dragendoff's | Orange brown ppt | + |

+ Test substance present; – Test substance absent

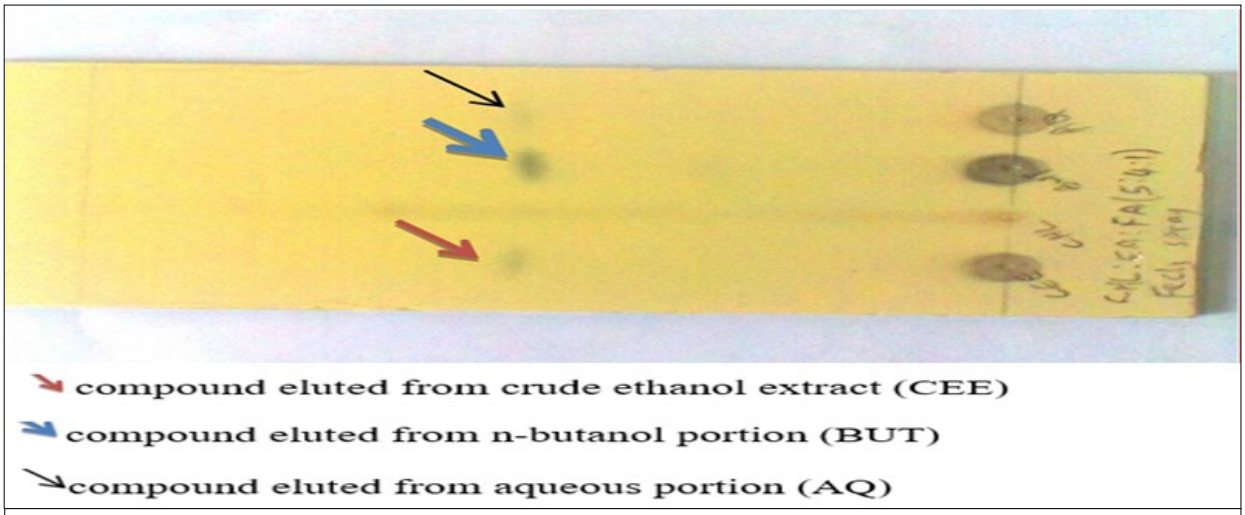


Figure 1. TLC plates showing chromatogram of different portions of leaf extracts of *A. montanus* under visible light.

Table 3. Mean (± SEM) worm count in mice infected with 150 $L_3$ of *H. bakeri* and orally treated for 5 consecutive days with varying doses of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

| **Dose (g/kg)** | **Substance** | | | | |
|---|---|---|---|---|---|
| | **CEE** | **BUT** | **AQ** | **DW (5 mL/kg)** | **ABZ (10 mg/kg)** |
| 1.2 | $10.40\pm0.51^{a}$ (5) | $6.67\pm0.33^{a}$ (3) | $12.67\pm0.88^{a}$ (3) | $20.25\pm1.11^{c}$ (4) | $0.00\pm0.00^{b}$ (4) |
| 1.4 | $8.20\pm0.86^{a}$ (5) | $4.20\pm0.37^{b}$ (5) | $10.80\pm0.58^{a}$ (5) | | |
| 1.7 | $7.20\pm0.58^{a}$ (5) | $1.80\pm0.49^{b}$ (5) | $8.40\pm0.51^{a}$ (4) | | |
| 2.0 | $5.60\pm0.33^{a}$ (5) | $0.60\pm0.40^{b}$ (5) | $7.50\pm0.65^{a}$ (4) | | |

F=78.043 (MStreatment/MSresidual)

5 mL/kg is the Maximum convenient volume (MCV)

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10 mg/kg is the Dose at manufacturer's recommendation

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a,b,c, differ significantly ($p<0.05$) from one another. Numbers in parenthesis show the number of mice up to the end of the experiment

Table 4. Percentage deparasitization (%) in mice infected with 150 $L_3$ of H. bakeri and orally treated for 5 consecutive days with varying doses of A. montanus leaf extracts, distilled water or albendazole 19 days post infection.

| Dose (g/kg) | Substance | | | | |
|---|---|---|---|---|---|
| | CEE | BUT | AQ | DW (5 mL/kg) | ABZ (10 mg/kg) |
| **1.2** | 48.64 | 67.06 | 37.43 | 0.000 | 100.0 |
| **1.4** | 59.51 | 86.17 | 46.67 | | |
| **1.7** | 64.44 | 91.11 | 58.52 | | |
| **2.0** | 72.35 | 97.04 | 62.96 | | |

5 mL/kg is the Maximum convenient volume (MCV)
10 mg/kg is the Dose at manufacturer's recommendation

The chromatograms of TLC plates spotted with the various portions of *A. montanus* sprayed with 1% $FeCl_3$ are shown in Plate I. *N*-butanol (BUT) portion displayed more conspicuous spot followed by crude ethanol extract (CEE), then the aqueous (AQ) portion. Chloroform [CHL(O)] portion did not display any visible spot (under visible light). The *Rf* value which is depicted as retardation factor, rate of flow or retention factor for each spot was calculated and the same value obtained. This indicates a particular compound (solute) being eluted. This result shows that the compound is polar considering the eluting system (mobile phase) and the stationary phase (silica gel). The colour of the spots was greenish black. This is an evidence of phenolics (likely tannins, because of 1% $FeCl_3$ spray reagent used) eluted.

$$Rf\ (retention\ factor) = \frac{distance\ travelled\ by\ compound\ (solute)}{distance\ travelled\ by\ solvent\ front} = \frac{3.9\ cm}{7.4\ cm} = 0.53$$

*Results of Anthelmintic Trials of Fractions of A. Montanus*

There was a dose-dependent decrease in worm count in mice given each of the three preparations as shown in Table 3. Thus, for each extract, the highest worm count was seen in mice given the lowest dose of 1.2 g/kg; and the lowest count in those given the highest dose of 2.0 g/kg. For each dose level, mice given the *n*-butanol portion had the lowest worm counts, followed by those given the crude ethanol extract; and then those given the aqueous portion.

The worm counts of the albendazole-treated (0.00±0.00) positive control group was not significantly different ($p>0.05$) from the counts (0.60±0.40) of mice treated with *n*-butanol extract at the highest dose level of 2.0 g/kg. The degree of deparasitization (%) achieved by dosing with the extracts or albendazole or distilled water is the direct converse of the data on worm count (Table 4).

The *n*-butanol extract gave the highest rate of deparasitization at the four doses that were used. At 2.0 g/kg dose rate, the deparasitization rate achieved through *n*-butanol was 97% compared to 72.4% and 63% for crude ethanol extract and aqueous portion respectively. The deparasitization caused by *n*-butanol exract at any of the doses was significantly higher ($p<0.05$) compared to those caused by either crude ethanol extract or aqueous portion at such corresponding dose.

## Discussion

*Acanthus montanus* was selected according to earlier reports of anthelmintic efficacy [17] and folkloric claims. Among the fractions tested, *n*-butanol extract produced profound anthelmintic activity. The degree of deparasitization obtained in this study is similar to that obtained from other studies with plants that were found to produce anthelmintic effects [25,24]. The results of the anthelmintic study indicate that *n*-butanol portion of *A. montanus* leaf produced significantly higher deparasitization followed by crude ethanol and then aqueous portions. The efficacy of the *n*-butanol extract

(portion), for instance increased with dose, an indication of graded response of the parasite to the drug [26,27]. This is probably due to the activity of tannins that was found to be more in the *n*-butanol extract (fraction). Different classes of anthelmintics are known to show profound effects on the physical activities, generally culminating into loss of mobility and mortality of helminth parasites in a dose-dependent manner [28]. The active principle(s) in the *A. montanus* extracts responsible for this anthelmintic activity might be individual phytocomponents as detected during phytochemical screening or a number of them working in synergy. Plant secondary metabolites found in this study have been reported to occur in other plants with anthelmintic activity [25,7]. In the present study, tannins (condensed tannins) were likely responsible for the observed profound anthelmintic activity [29] due to its abundance in the extract screened phytochemically and confirmed by thin layer chromatography. Chemically, tannins are polyphenolic compounds which are uncouplers of oxidative phosphorylation in helminth parasites [30]. Some synthetic phenolic anthelmintics as niclosamide, oxyclozanide and bithionol, are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [31]. It is possible that the large amount of tannins detected in the *n*-butanol leaf extracts of *A. montanus* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal [32] or glycoprotein on the cuticle of the parasite [33], and may thus cause death.

Polyphenolic compounds which are reported to be present in leaves of mainly dicotyledonous plants are potent anthelmintics. Condensed tannins which are derived from flavonol are soluble in water and are capable of precipitating proteins. They are reported to be found in cell walls or stored in vacuoles, stems, leaves, flowers or seeds [34,35]. Tannins protect the intestine from reinfection by "tanning" proteins in the lining of the gut (intestine) [36]. Consumption of vegetative portions of such plants is advocated. This may explain why the plant's (*A. montanus*) aerial parts are used as vermifuge by the Etulo natives of Benue State, Nigeria. Its tannin content may explain its anthelmintic activity.

## Conclusion

This study has demonstrated that the *n*-butanol extracts of *Acanthus montanus* leaf has profound anthelmintic activity against experimental *Heligmosomoides bakeri* infection in mice. It is therefore recommended that further investigations should be carried out on *A. montanus* leaf extracts to explore it commercial potential in the treatment of helminths of animals and humans. Also, detail evaluation of the significance of retention factor *(Rf)* of tannins from Thin layer chromatography of 0.53 is to be investigated. The secondary plant metabolites detected from *A. montanus* leaf might be responsible for anthelmintic activities singly or in synergy especially the *n*-butanol extract.

## Conflict of Interest

The authors declare that they do not have conflict of interest.

## References

1. Hammond, J.A., Fielding, D. and Bishop, S.C. (1997). Prospects for plant anthelmintics in tropical Veterinary Medicine. *Veterinary Resource Communication,* 21: 213-228.
2. Perry, B.D. and Randolph, T.F. (1999). Improving the assessment of the economic impact of parasitic disease and of their control in production animals. *Veterinary Parasitology,* 84:145-168. Publishing Ltd., London, pp. 196-197.
3. McGaw, L.J. and Eloff, J.N. (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology,* 119: 559-574.
4. Behnke, J. and Harris, P.D. (2010). *Heligmosomoides bakeri: a* new name for an old worm? *Trends in Parasitology,* 26(11): 524-529.
5. Al-Bassel, D. A.; Stietieh, F. M. and Farrag, A. M. (2000). On the morphology of *Heligmosomoides polygyrus* (Nematoda-Trichostrongylidae) from the field mouse *Apodemus sylvaticus*. *Journal of the Egyptian Society of Parasitology*. 30 (1): 43–49. ISSN 1110-0583. PMID 10786017.
6. Pleass, R. J.; Bianco, A. E. (1995). The effects of gamma radiation on the development of *Heligmosomoides polygyrus bakeri* in mice. *International Journal for Parasitology*. 25 (9): 1099–1109. doi: 10.1016/0020-7519(95)00010-

Y. PMID 8847171.

7. Wabo, P.J., Tankoua, O.F., Yondo, J., Komtangi, M.C., Mbida, M. *et al*. (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). *Veterinary Medicine International,* 1.
8. Enejoh, S.O., Shuaibu, K., Suleiman, M.M. and Ajanusi, J.O. (2014). Evaluation of anthelmintic efficacy of *citrus aurantifolia* (christm) fruit juice against *heligmosomoides bakeri*. *International Journal of Advanced Biological Research,* 4(4): 448-45.
9. Belemlilga, M.B., Traoré, A., Belemnaba, L., Kini, F.B., Sylvin Ouédraogo, S. *et al*. (2019). Ovicidal and Larvicidal Activities of *Saba senegalensis* (ADC) Pichon (Apocynaceae) Extracts and Fractions on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). *Journal of Pharmaceutical Research International,* 1-13. DOI: 10.9734/ jpri/2019/v31i630327
10. Gregory, R.D., Keymer, A.E., Clarke, J.R. (1990). Genetics, Sex and Exposure: The Ecology of *Heligmosomoides polygyrus* (Nematoda) in the Wood Mouse. *Journal of Animal Ecology*. 59 (1): 363–378. doi:10.2307/5178.
11. Githiori, J.B., Höglund, J., Waller, P.J. and Baker, R.L. (2003). The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. *Veterinary Parasitology*, 116(1): 23-34.
12. Eguale, T., Tilahun, G., Debella, A., Felake, A. and Makonnen, E. (2007). *In vitro* and *in vivo* anthelminthic activity of crude extracts of *Coriandrum sativum* against *Heamonchus contortus. Journal of Ethnophaemacology,* 110: 428-433.
13. Huxley, A.J. (1992). The New Royal Horticultural Society Dictionary of Gardening. London: Macmillan; *Acanthus montanus* plant description and geographical distribution. http:// wwwdoacs.state.flaus/pi/enpp/98mar.apr.htm.
14. Sanganuwan, S.A. (2009). Tropical plants with antihypertensive, antiasthmatic and antidiabetic value. *Journal of herbs, spices and medicinal plants,* 15: 24-44.
15. Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A. and Igoli, N.P. (2005). Traditional Medicine Practice amongst the Igede people of Nigeria Part II. *African Journal Traditional Complimenary and Alternative Medicines,* 2(2): 134-152.
16. Agishi, E.C. (2004). *Etulo, Idoma, Tiv and Hausa names of plants.* Agitab Pub. Ltd. Makurdi.
17. Adamu, M., Oshadu, O.D. and Ogbaje, C.I. (2010). Anthelmintic efficacy of aqueous extract of *Acanthus montanus* leaf against strongylid nematodes of small ruminants. *African Journal of Traditional, Complementary and Alternative Medicine,* 7(4): 279 -285.
18. Evans, W.C. (ed) (2002). Trease and Evans "Pharmacology" 15th Edition, W.B. Saunders, New York, pp. 221-393.
19. Mehta, S. (2012). Thin Layer Chromatography (TLC): Principles (with Animation), On-line [Accessed May 11, 2012].
20. Harborne, J.B. (1984). Phytochemical methods: a guide to modern techniques of plant analysis, 2nd edition. London: Chapman and Hall.
21. Burren, C.H. (1980). *Zeitschrift fur Parasitenkunde*, 62: 111-112.
22. Lai, S.C. (2006). Chinese herbarbal medicine Yin-Chen extract as adjunt to anthelmintic albendazole used against *Angiostrongylus cantonensis*-induced eosinophilic meningitis or meningoencephalitis. *American Journal of Tropical Medicine and Hygiene,* 75(3): 556-562.
23. Johnston, C.J., Robertson, E., Harcus, Y., Grainger, J.R., Coakley, G. *et al.* (2015). Cultivation of *Heligmosomoides polygyrus:* An Immunomodulatory Nematode Parasite and its Secreted Products. *Journal of Visualized Experiments,* (98), e52412, doi:10.3791/52412
24. Jegede, O.C., Agbede, R.I.S., Ajanusi, J.O. and Adaudi, A.O. (2009). Anthelmintic screening of fractions of *Spigelia anthelmia* Linn. extracts against experimental *Nippostrongylus brasiliensis* in rats. *Veterinarski Arhiv,* 79(2): 189-197.
25. Suleiman, M.M., Mamman, M., Aliu, Y. O., Ajanusi, J.

O. and Abubakar, M. S. (2005). Anthelmintic Effect of Extracts of *Terminalia avicennioides* Against Experimental Nippostrongylosis in Rats, *Journal of Herbs, Spices, and Medicinal Plants,* 11(3): 117–126.

26. Iqbal, Z., Lateef, M., Jabbar, A., Muhammad, G. and Khan, M.N. (2006a). Anthelmintic activity of *Vernonia anthel* seeds against *Trichostrongylid* nematodes of sheep. *Pharmaceutical Biology,* 44(8): 563-567.
27. Iqbal, Z., Lateef, M., Jabbar, A., Muhammad, G and Gilani, A.H. (2006b). *In vitro* anthelmintic activity of *Nicotiana tobacum* Linn. leaves against gastrointestinal nematodes of sheep. *Phytotherapy Research,* 20(1): 46-48.
28. Xiao, S.H., Guo, J., Chollet, J., Wu, J.T., Tarma, M. *et al*. (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.
29. Niezen, J.H., Waghorn, G.C., Charlestone, W.A.G. and Waghorn, G.C. (1995). Growth and gastrointestinal parasitism in lambs grazing either Lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*), which contains condensed tannins. *Journal of Agricultural Science,* 125: 281-289.
30. Bate-Smith, E.C. (1962). The phenolic constituents of plants and their taxonomic significance, Dicotyledons. *Journal of Linn Society Botany,* 58: 95-117.
31. Martin, R.J. (1997). Mode of actions of anthelmintic drugs. *Veterinary Journal,* 154: 11-34.
32. Athanasiadou, S., Kyriazakis, I., Jackson, F. and Coop, R.L. (2001). Direct anthelmintic effect of condensed tannins towards different gastrointestinal nematodes of sheep: *In vitro* and *in vivo* studies. *Veterinary Parasitology,* 99: 205-219.
33. Thompson, D.P. and Geary, T.G. (1995). The structure and function of helminth surfaces. *In:Biochemistry and Molecular Biology of parasites*, Marr, J.J. (Ed), Academic Press New York, 1st edition, 1995, pp. 203-235.
34. McMahon, R.T., McAllister, T.A., Berg, B.P., Majiak, W., Acharya, S.N. *et al*. (2000). A review of effects of forage condensed tannins on ruminal fermentatation and bloat in grazing cattle. *Canadian Journal of Plant Science,* 80: 469-485.
35. Acuna, H., Concha, A. and Figueroa, M. (2008). Condensed tannins concentrations of three *Lotus* species grown in different environments. *Chilean Journal of Agricultural Research,* 68(1): 31-41.
36. Wallace, D. (2012). Papaya power! (which includes papaya enzymes to aid in gluten digestion). *Eat Smart, The Eating Revolution.* http:/ www.gaiahealthblog.com (Online Article accessed on 5th May, 2021).