Agrotechnology
ISSN: 2168-9881

Simon and Jegede, Agrotechnol 2013, 2:2
DOI: 10.4172/2168-9881.1000111

Research Article Open Access

Phytochemical Screening and Anthelmintic Evaluations of the Stem Bark of *Afzelia Africana* 'SM' (Keay, 1989) against *Nippostrongylus Barziliensis* in Wistar Rats

Simon MK* and Jegede CO

Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Abuja, Nigeria

Abstract

The anthelmintic activity of partitioned portions of the crude methanolic extract of *Afzelia africana* was evaluated *in-vivo* in rat model, experimentally infected with *Nippostrongylus braziliensis*. Crude methanolic extract of the plant was obtained and further partitioned between three solvents (petroleum ether, chloroform and N-butanol). Four portions (i.e., petroleum ether, chloroform, N-butanol and the aqueous methanol portions) were obtained after the partitioning. The crude methanolic extract and all the portions (with the exception of petroleum ether) were tested for anthelmintic activity against *N. braziliensis* in rats. The anthelmintic activity was assessed by comparing the number of worms recovered from rats treated with the portions to those from non-treated infected controls. Deparasitization rate of 70% or greater was considered as significant. The chloroform and N-butanol portions produced significant deparasitization ($p<0.05$) when data were subjected to ANOVA. The chloroform and N-butanol portions caused deparitization at the rate of 79.20% and 72.72% respectively when a maximum tolerated dose (1000 mg/kg^{-1}) was administered. The crude methanolic and aqueous methanol extracts induced non-significant ($p>0.05$) deparasitization rate of 62.50% and 53.24% respectively. Phytochemical screening conducted on the crude methanolic extract and the four portions of the plant revealed constituents that has anthelmintic activity such as alkaloids; steroids, saponins, tannins, flavonoids and cardiac glycoside.

Keywords: In-vivo; Phytochemistry; *Anthelmintic; Afzelia africana; Nippostrongylus braziliensis*

Introduction

Parasitic nematodes are among the most common and economically important agents of infectious disease of grazing livestock, especially in small ruminants; in the tropics, subtropics and other parts of the world [1,2]. Livestock production in tropical climates suffers heavy economic losses due to gastrointestinal parasites [3,4]. The greatest losses associated with nematode parasite infections are sub-clinical, and economic assessment shows that the financial costs of internal parasitism are enormous [5,6]. The loss is characterized by lower output of animal products (meat, milk, hides and skins), low manure, poor traction power, poor carcass quality/organ condemnation, death and medication costs, which all impact negatively on the livelihood of small holder farmers [7,8].

Livestock producers have generally derived substantial benefit from the use of conventional anthelmintic drugs in controlling livestock parasitosis. In Africa, however, declining funding for veterinary services and the rising costs (occasioned by depreciating value of local currencies) of these services has made it difficult for resource-poor farmers to have access to such services. This has led to the increasing demand for effective and low cost anthelmintic drug by African smallholder livestock producers and pastoralist in order to reduce having to pay for the high cost of the services [9-12]. There is equally the need to produce animal products free from industrial chemical input [13,14] and avoid possible tendencies of environmental pollution [15]; also the need to discover new therapeutic substances of natural origin with low toxicity to man and animals [16], as well as overcoming the rapid escalation in anthelmintic resistance worldwide [17]. *Afzelia africana SM* is a tree species commonly found in savanna fringing forest and drier parts of forest regions. It is commonly referred to as; mahogany, kawo, apa and akpalata in English, Hausa, Yoruba and Igbo respectively [18]. The seed is widely used for medicinal purposes, for industrial production of soap, margarine, and candle making and as diets such as condiments and seasoning in soup [19]. Atawodi [20] identified the use of herbal preparations including *Afzelia africana* in the treatment of helminthiasis. This study therefore asseed the *in vivo* anthelmintic effects of the crude methanolic extract and partitioned portions of the crude methanolic extract of the bark of *Afzelia africana* against adult *Nippostrongylus braziliensis* in experimentally infected rats and also identified the active fractions from the crude methanolic extracts of the plant via a separation process, with the view of providing scientific basis for their use in ethno-veterinary practices.

Materials and Methods

Plant collection, identification and preparations

The stem barks of *Afzelia africana* was collected in April, 2005 from New Bussa in Niger State, Nigeria. The plant was identified by a botanist from the department of biological sciences Ahmadu Bello University, Zaria and a specimen voucher number 2276 was deposited. The samples (5 kg) of the plant bark were sun dried, pulverized into powdered form using mortar and pestle and sieved as described by Onyeyili et al. [21]. The methanolic extract was obtained using 500 g the powdered plant material in a soxhlet apparatus (Quick fit corning Ltd, Stafford England) after which the solution was evaporated to dryness in vacuum using a rotary evaporator coupled to a thermo-regulator (RII-35). Twenty grammes (20 g) of the dried crude methanolic extract were partitioned

***Corresponding author:** Simon MK, Department of Veterinary Parasitology and Entomology, University of Abuja, Nigeria, E-mail: kawesimon2002@yahoo.com

Received April 27, 2013; **Accepted** April 29, 2013; **Published** July 15, 2013

Citation: Simon MK, Jegede CO (2013) Phytochemical Screening and Anthelmintic Evaluations of the Stem Bark of *Afzelia Africana* 'SM' (Keay, 1989) against *Nippostrongylus Barziliensis* in Wistar Rats. Agrotechnol 2: 111. doi:10.4172/2168-9881.1000111

with petroleum ether, chloroform and N-butanol (150 ml each) using separating funnel as described by Suleiman et al. [22]. The portions were then referred to as petroleum ether, chloroform, N-butanol and aqueous methanol portion respectively. After evaporating the solvents, the portions were tested for anthelmintic activity on albino rat experimentally infected with L3 stage of *Nippostrongylus braziliensis*.

Phytochemical screening

Before the experimental treatment, the crude methanolic extract, petroleum ether, chloroform, N-butanol and the aqueous methanol portions of the extracts were subjected to phytochemical screening using standard techniques of Ciulei [23]; Sofowora [24] and Brain and Turner [25]. The three solvents used have different polarity, thus, it is expected that they extract the various active principles in the plant base on their polarity.

Experimental animal

Sixty three (63), six to seven weeks old albino Wistar rats of both sexes and weighing between 100 to 160 g were used. The rats were acclimatized for two weeks in the laboratory and fed commercially prepared feed; water was given ad lib. Rats for the anthelmintic study were dewormed using albendazole at 200 $mgkg^{-1}$ two weeks before infection [22].

Toxicity test

Due to dearth of information on the precise dosage of the plant preparation, a maximum tolerated dose experiment described by Lorke [26] was determined using fifteen (15) rats. The established maximum tolerated dose was then used as the basis for the administration of the crude methanolic extract and the various portions of the plant extract in the anthelmintic activity studies.

Experimental infection/design

Forty two worm-free rats were infected subcutaneously in the cervical region with 200 viable L3 stage of *N. braziliensis* in 0.2 ml of distil water using an 18-gauge needle attached to an insulin syringe [22]. Five days post infection, fresh faecal sample from each infected rat was collected by squeezing it out from the rectum. Faecal samples were examined quantitatively for *N. braziliensis* egg using the simple floatation method [27]. Rats not shedding ova of *N. braziliensis* were excluded from the experiment. The infected rats were randomly allocated to three (3) experimental groups (A-C). Group A (positive control group) having six rats, were treated with albendazole at 200 $mgkg^{-1}$ body weight [22], Group B (experimental group) having twenty four rats; were divided into four sub-groups of six rats each and treated with the crude methanolic, chloroform, N-butanol and aqueous methanol portions of the extract based on the maximum tolerated dose [28]; whereas group C (negative control) having twelve rats; were subdivided into two groups of six rats each and given distil water and propylene glycol as placebo based on the maximum convenient volume (MCV) of 5 $mlkg^{-1}$ [22].

Treatment with crude methanolic extract and the various portions

Oral treatment with the crude methanolic extract and the various portions of the plant extract was administered on day seven (7) post infection. Before the treatment all rats were weighed to determine the appropriate dose and the maximum convenient volume (MCV) for individual rats. Observation was made daily for two days for abnormal behavioral signs.

Worm counts

Two days post treatment; the treated rats were fasted for 24 hours, salvaged for adult worm count using the WAAVP guides [29]. The first 15 cm of the small intestine was removed, cut longitudinally and placed between two clean 20 cm glass slides. The section was examined at 40X magnification of a dissecting microscope. Visible worms were counted and recorded. The fraction that caused the highest reduction in worm count and does not produce any behavioral changes in the rats was considered to be the most active portion.

Percentage deparasitization

Percentage efficacy (deparasitization) of the crude methanolic extract and the various portions of was calculated according to the method used by Cavier [30].

Statistical analysis

Means of data obtained were analyzed statistically using the software package for GraphPad prism (version 4.0-----2003). Statistical significance for the anthelmintic effect of crude methanol extract, chloroform, butanol portion and aqueous portion was assessed by ANOVA; subsequently Borferroni's multiple comparison tests. P value <0.05 was considered significant.

Results

Phytochemical screening

The crude methanolic extract of the plant had alkaloids, steroids, saponins, carbohydrate, flavonoids, tannins and cardiac glycosides as constituents; the petroleum ether portion of the plant revealed the presence of only alkaloids and steroids; while the chloroform portion, when screened, showed the presence of alkaloids, steroids, flavonoids, tannins and cardiac glycosides. The screening of the N-butanol portion revealed the presence of carbohydrate, in addition to the constituent seen in the chloroform portion. The screening of the aqueous methanol portion revealed the presence of steroids, flavonoids, tannins, carbohydrate and cardiac glycosides (Table 1).

Toxicity test

At a dose range of 10 to 1000 $mgkg^{-1}$, the crude methanol did not cause any visible toxic effect on the rats. On the other hand, from 1600 to 5000 $mgkg^{-1}$, the rats demonstrated varying degrees of signs of toxicity which manifested as visible body weakness, inability to move and reduced appetite from 24 hrs to 7 days post administration. Therefore the doses of 1600 to 5000 $mgkg^{-1}$ were considered unsafe for the rats; and the dose of 1000 $mgkg^{-1}$ body weight was chosen as the experimental treatment dose for both the crude methanol extracts and the various portions (Table 2).

Constituents	CME	Portions Pet ether	Chloroform	N-butanol	Aq. Methanol
Alkaloids	+	+	+	+	-
C. glycoside	+	-	+	+	+
Carbohydrate	+	-	-	+	-
Flavonoids	+	-	+	+	+
Saponins	+	-	-	+	+
Steroids	+	+	+	+	+
Tannins	+	-	+	+	+

Table 1: Phytochemical screening for the CME and various partitioned portions of *A. africana*.

	10	100	Dose (mg/kg^{-1}) 1,000	1,600	2,900	5,000
Initial No. of rat	3	3	3	3	3	3
Mortality	0	0	0	0	0	0
Observation	A	A	A	B	C	D
Inference	-	-	-	+	++	+++

Key
A=rats active 6-24 hrs and beyond B=rats showed weakness for more than 24 hrs C=rats showed weakness for more than 48 hrs D=rats showed weakness for more than 7 days
-=no sign of toxicity +=slightly toxic ++=less toxic +++=toxic

Table 2: Maximum tolarated dose/toxicity of crude methanol extract of *A. africana* on rats.

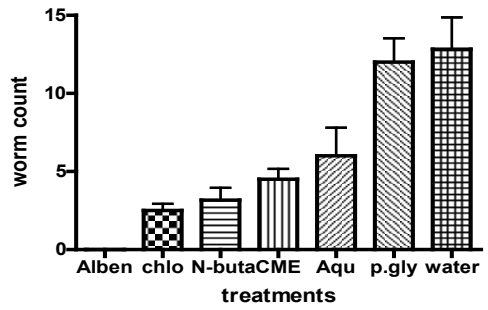

Figure 1: Mean ± SD of worm count after treatment with the various fractions of *A. africana* extracts, albendazole and the placebos.
alben-albendazole, **CME**-crude methanol extract, **N-but**-N-butanol, **Chlor**-chloroform, **Aqu**-aqueous methanol, **P.glycol**-propylene glycol.

The anthelmintic effect of the crude methanolic extract and the various portions

Rats that had oral infection of 200 L3 followed by treatment with crude methanolic extract at 1000 mg/kg^{-1} had a mean worm count of 4.5; while those infected and treated with chloroform, N-butanol and aqueous methanol portion at 1000 mg/kg^{-1} and albendazole at 200 mg/kg^{-1} had respective mean worm count of 2.5, 3.5, 6.0 and 0; compared to the mean worm count 12.83 and 12.0 from the negative controls (water and p. glycol) treated rats (Figure 1). The crude methanol extract and the respective portion produced percentage deparasitization of 62.50% (crude methanol extract), 79.20% (chloroform), 72.72% (N-butanol) and 71.40% (aqueous methanol). Albendazole-treated rats (positive control), gave a 100% deparasitization, while water and p. glycol (negative controls) gave 0% deparasitization (Table 3). The deparasitization produced by the crude methanol extract and the chloroform, N-butanol and aqueous methanol fractions were significant ($p<0.05$) when compared to that produced by the placebo-treated negative control rats, while the deparasitization produced by the albendazole extract was highly-significant ($p<0.001$) (Table 3).

Discussion

Recent harmonizations on anthelmintic efficacy guidelines in ruminants have indicated that for a drug to be considered efficacious, a 90% reduction in total worm count (TWC) should be achieved [28]. However, it was considered 'a priori' that the efficacy of the plant extracts would be biologically significant if a reduction in total worm count (TWC) above 70% occurred [31]. Rats treated with chloroform and N-butanol portions of the plant showed anthelmintic activity. The chloroform had the highest reduction in TWC of 79.20%; this was followed by N-butanol portion with reduction in TWC by 72.20%. The crude methanolic extract and aqueous portion did not produce the required biological significant reduction in TWC (62.50% and 53.24% respectively). However, the crude methanolic extract was statistically significant in comparison with the untreated control groups. The results of this study equally demonstrated that the parasite *N. braziliensis* was highly sensitive to albendazole with complete deparasitzation (100%) at a dose rate of 200 mg/kg^{-1} body weight; this is similar to the findings reported by Suleiman et al. [22]. The N-butanol and aqueous portions were soluble in water and other polar solvents like alcohol, suggesting that the constituents of these portions are mainly polar compounds. However, the crude methanol extract and chloroform fractions were only soluble in propylene glycol (a non polar solvent). The outcome of the phytochemical screening revealed that the plant had constituents including tannins, alkaloids, flavovoids, cardiac glycosides and sterols which may have helminthic activities [14,21,32-37]. Tannins have been shown to have anthelmintic activities [32]. However, the anthelmintic effect of plants containing tannins depends on the type and content of tannins in the plant [32,38]. For instance, Kahiya et al. [39] in *in-vitro* studies reported that condensed tannins from the leave extract of *Acarcia nitotica* inhibited the development of *H. contortus* larvae in goat. In another study, tannins polyphenols from bryophytes were shown to have anthelmintic activity against *N. braziliensis* [34]. Tannins could binds to the free proteins available in the GIT of the host reducing nutrients available to the parasites resulting into starvation and death [40,41]. Also tannins are capable of binding with the glycoproteins on the cuticle of the parasites leading to their death of the parasite [42]. It is therefore probable to assume that the tannins contained in this plant could have had similar anthelmintic effect with the ones earlier described. Flavonoids are also believed to stimulate intestinal motility similar to that produced by acetylcholine [43], thereby causing rapid worm expulsion from the GIT. Lahlou [36] reported that flavonoid is one of the phytochemicals that have anthelmintic effect. Having identified flavonoids in almost all the portions used in this study, it is possible that it has had a significant anthelmintic effect on the *N. braziliensis* resulting in the observed deparasitization.The present study has shown that alkaloids are present in all the portions. It is possible that the presence of alkaloids has contributed to the significant deparasitization observed. Previous findings by Al-Qarawi et al. [44] reported that alkaloids extracted from both the latex and leaves of *Calotropis procera*, was effective in inhibiting the exsheatment of L_3 of *H. contortus* to L_4 in sheep, while Lateef et al. [37] also reported that alkaloids and their glycosides extracted from the root of *Adhatoda vestica* was effective against mixed gastrointestinal infections in sheep. Cardiac glycoside was identified and prominent in all the portions used in this study and therefore may have contributed to the observed anthelmintic effect of the plant. Cardiac glycoside has been shown to induce tonic contraction that resulted in the expulsion of the worms from rat GIT [37,45-47]. The dose determination studies (maximum tolerated dose) [28] was carried out on the premise that the plant extracts under investigation had nmgkg^{-1}) o alternative data to support any intended dosage. In this work, the injurious dose (1600 to 5000 and the MTD (1000 mgkg^{-1}) were determined. However, the plant extracts could not produce death even when the highest dose (5000 mgkg^{-1}) was given; thus suggesting the safety of the extract. The investigation of chemical compounds from natural products is fundamentally important for the development of new anthelmintic drugs, especially in view of the vast worldwide flora. Thus a quality controlled extraction of *A. africana* and the isolation of their bioactive compounds could be a promising alternative to conventional anthelmintic for the treatment of gastrointestinal helminthes of ruminant in the future. One problem associated with the use of these plants in traditional medicine is lack of

Rats	CME (1000 mgkg^{-1})	Worm count after treatment with chloroform (1000 mgkg^{-1})	N-buta (1000 mgkg^{-1})	Aq. M (1000 mgkg^{-1})	Alb. (1000 mgkg^{-1})	Placebo1 (1000 mgkg^{-1})	Placebo2 (1000 mgkg^{-1})
1	4	2	4	14	0	4	6
2	2	3	3	4	0	17	16
3	7	4	2	8	0	18	15
4	5	1	5	3	0	12	10
5	5	3	1	5	0	14	11
6	4	2	6	2	0	12	14
Mean ± SD % DPZ	4.5 ± 1.64b* 62.50	2.5 ± 1.05a* 79.20	3.5 ± 1.87a* 72.72	6.0 ± 4.43b 53.24	0.0 ± 0.0a* 100	12.83± 5.0 0	12.0 ± 3.74 0

Key
CME=crude methanol extract, Aq.M=aqueous methanol, Alb=albendazole,
Mean with *within the column are significantly different at p<0.001, while those with the letter a and b show no significant difference between their means at p>0.05 as determined by Borferroni's multiple comparison test.
%DPZ=percentage deparasitization

Table 3: Worm count and percentage deparasitization 7 days after treatment with crude methanol extract and fractions of *A. Africana.*

consistency of the dose. However, this was overcome by evaluating a maximum tolerated dose in order to reveal an appropriately non-toxic dose that was used in this study.

Conclusion

Result from this study demonstrated that the chloroform and N-butanol (partition portions of CME) of the plant are effective against experimental *N. braziliensis* infection in rats at a non-toxic dose of 1000 mgkg^{-1}. The chemicals believed to constitute the active principles in *A. africana* have significant anthelmintic efficacy whereas albendazole was found to be highly efficacious. However, the efficacies of these portions have no statistical significant difference to those of albendazole at a dose rate of 200 mgkg^{-1}. This demonstrates that the rat model was a reliable system for assessing *in-vivo* anthelmintic efficacy [48]. The result obtained in this study justifies further investigation into anthelmintic effects of the two portions of the plant extract in other animal species. More detailed studies are needed to isolate, characterized and evaluate the active components and the mechanism of action.

Acknowledgements

The authors thank the University of Abuja, Nigeria for granting the main author a staff development leave. The authors also like to thank all the technical staff of the Department of Veterinary Parasitology and Entomology for their assistance.

References

1. Prichard R (1994) Anthelmintic resistance. Vet Parasitol 54: 259-268.
2. Perry BD, Randolph TI, McDermoh JJ, Sones KR, Thornton PK (2002) Investing in animal health research to alleviate Poverty. International livestock research institute (ILRI) Kenya, 148.
3. Copeman DB (1980) The importance of helminth parasites in animal production system in the tropics [ruminants; South East Asia]. Asian-Australasian Animal Science Congress, Selangor, Malaysia.
4. Al-Quaisy HH, Al-Zubaidy AJ, Altaf KI, Makkwai TA (1987) The pathogenicity of haemonchosis in sheep and goats in Iraq: 1. clinical, parasitological and haematological findings. Vet Parasitol 24: 221-228.
5. Preston JM, Allonby EW (1979) The influence of breed on the susceptibility of sheep of Haemonchus contortus infection in Kenya. Res Vet Sci 26: 134-139.
6. McLeod RS (1995) Costs of major parasites to the Australian livestock industries. Int J Parasitol 25: 1363-1367.
7. Perry BD, Randolph TF (1999) Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet Parasitol 84: 145-168.
8. Chiezey NP, Gefu JO, Jagun AG, Abdu PA, Alawa CBI, et al. (2000) Evaluation of some Nigerian plants for anthelmintic activity in young cattle. Proceedings of International Workshop on Ethnoveterinary Practices, Kaduna, Nigeria.
9. Uza DV, Umunna NN, Oyedipe EO (1996) The productivity of muturu cattle (*Bos brachycerus*) under the traditional management system. Herd health. Bulletin of Animal Health and Productin in Africa 444: 151-152.
10. Sangster NC (1999) Anthelmintic resistance: past, present and future. Int J Parasitol 29: 115-124.
11. Abdu PA, Jagun AG, Gefu JO, Mohamme AK, Alawa CBI et al. (2000) A survey of ethnoventrinary practices of agropastoralist in Nigeria. In: Gefu, JO, Abdu PA, Alawa CBI (Eds) Ethnovet practices in Nigeria. Proceeding of the International workshop on ethnoveterinary practices Kaduna, Nigeria.
12. Ademola IO, Fagbemi BO, Idowu SO (2004) Evaluation of the anthelmintic activity of Khaya senegalensis extract against gastrointestinal nematodes of sheep: in vitro and in vivo studies. Vet Parasitol 112: 151-164.
13. Githiori JB, Höglund J, Waller PJ, Leyden Baker R (2003) Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. Vet Parasitol 118: 215-226.
14. Athanasiadou S, Tzamaloukas O, Kyriazakis I, Jackson F, Coop RL (2005) Testing for direct anthelmintic effects of bioactive forages against *Trichostrongylus colubriformis* in grazing sheep. Vet Parasitol 127: 233-243.
15. Vierra LS, Calvalcante ACR, Pereira MF, Dantas LBA, Ximenes LJF (1999) Evaluation of anthelmintic efficacy of plants available in Ceara State west east Brazil. For the control of goat gastro intestinal nematodes. Review of Veterinary Medicine 150: 447-452.
16. Guarrera MP (1999) Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. J Ethnopharmacol 68: 183-192.
17. Jackson F, Coop RL (2000) The development of anthelmintic resistance in sheep nematodes. Parasitology 120 (suppl) s95-s107.
18. Keay RWY (1989) Tress of Nigeria. (3rdEdn) Clavendon press Oxford 146-216.
19. Ajah PO, Madubuike FN (1997) The proximate composition of some tropical legume seeds grown in two states in Nigeria. Food Chem 59: 361-365.
20. Atawodi SE, Usman M, Bulus ST, Atawodi JC, Wakawa L, et al. (2000) Herba treatment of some peotozoan and parasitic diseases of poultry in the middle of Nigeria. Procceedings of International Workshop on Ethnoveterinary Practice 79-84.
21. Onyeyili PA, Amin JD, Gambo HI, Nwosu CO, Jibike GI (2001) Toxicity and anthelmintic efficacy of ethanolic stem bark extract of Nauchlea latifolia. Nigeria Veterinay Journal 22: 74-79.
22. Suleiman MM, Mamman M, Aliu YO, Ajanusi JO (2005) Anthelmintic activity of the crude methanolic exract of Xylopia aethiopica against Nipposstrongylus braziliensis in rats Veterinarski arhiv 75: 487-495.
23. Ciulei T (1981) Methodology for the analysis of vegetable drugs. Chemical industries branch. Division of Industrial Operation, UNIDO 16-30.
24. Sofowora A (1982) Medicinal plants and traditional medicine is Africa. John Wiley and Sons. NewYork. 76-77.
25. Brain KR, Turner TD (1975) The practical evaluation of phytopharmaceuticals and therapeutics. Wright-Scientechnica, Bristol 10-30.

26. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54: 275-287.

27. Soulsby EY (1982) Helminths, Arthropods and protozoa of domestic animals 7th (Edn.) FLBS Barrierve Tindal London.

28. Vencruysse J, Holdsworths P, Letonja T, Barth D, Conder, G, et al. (2001) International harmonization of anthelmintic efficacy guidelines. Vet Parasitol 99: 171-193.

29. Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T, et al. (1982) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet Parasitol 10: 265-284.

30. Cavier R (1973) Chemotherapy of internal nematode. In: Hawking F Chemotherapy of Helmithiasis (Edn.) International Encyclopedia of Pharmacology and Therapeutics. (1stEdn) Pergamon Press Ltd Headington Hill Hall, Oxford 1: 437-500.

31. Githiori JB, Hoglund J, Waller PJ, Baker RL (2003) The anthelmintic efficacy of the plant, Albizia anthelmintica, against the nematode parasites Haemonchus contortus, of sheep and Heligmosomoides polygyrus of mice. Vet Parasitol 116: 23-34.

32. Niezen JH, Waghorn GC, Charleston WAG (1998) Establishment and fecundity of *Ostertagia circumcinta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedenculanis*) or perenial rye grass (*Lolium perenne*). Vet Parasitol 78: 13-21.

33. Athanasiadou S, Kyriazakisi I, Jackson F, Coop RL (2000) Consequences of long-term feeding with condensed tannins on sheep parasitised with *Trichostrongylus colubriformis*. Int J Parasitol 30: 1025-1033.

34. Gamenara D, Pandolfi E, Saldaña J, Domínguez L, Martínez MM, et al. (2001) Nematocidal activity of natural polyphenols from bryophytes and their derivatives. Arzneimittelforschung 51: 506-510.

35. Prashanth D, Asha MK, Amit A, Padmaja R (2001) Anthelmintic activity of Butea monosperma. Fitoterapia 72: 421-422.

36. Lahlou M (2002) Potential of Origanum Compactum as a cercaricide in Morocco. Ann Trop Med Parasitol 96: 89-90.

37. Lateef M, Iqbal Z, Khan MN, Akhtar MS, Jabbar A (2003) Anthelmintic activity of *Adhatoda vesica* roots. Int J Agric Biol 5: 86-90.

38. Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC (1995) Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J Agric Sci 125: 281-289.

39. Kahiya C, Mukaratirwa S, Thamsborg SM (2003) Effects of Acarcia nilotica and Acacia karoo diets on Haemonchus contortus infection in goats. Vet Parasitol 115: 265-274.

40. Athanasiadou S, Kyriazakisi I, Jackson F, Coop RL (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep. In vitro and in vivo studies. Vet Parasitol 99: 205-219.

41. Schultz JC (1989) Tannins-insect interaction. In: Hemingway RW, Karchesy J.J (Ed.), Chemistry and significance of condensed tannins. Plenum Press New York 417-433.

42. Thompson DP, Geary TG (1995) The structure and fuction of helminth surfaces. In: Marr JJ (Ed.), Biochemistry and Molecular Biology of Parasites (1st edn), Academic Press. New York 203-232.

43. Akendenque B (1992) Medicinal plants used by the Fang traditional healers in Equatorial Guinea. J Ethnopharmacol 37: 167-143.

44. Al-Qarawi AA, Mahmoud OM, Sobaih, Haroun EM, Adam SE (2001) A preliminary study on the anthelmintic activity of Calotropis procera latex against Haemonchus contortus infection in Najdi sheep. Vet Res Commun 25: 61-70.

45. Kim YK, Valdivia HH, Maryon EDB, Anderson P, Coranado R (1992) High molecular weight proteins in nematode C. elegans bind (H) ryanodine and form a large conductance channel. Biophy J 63: 1379-1384.

46. Hong SJ (1996) Inhibition of mouse neuromuscular transmission and contractile function by okadaic acid and canthasridin. Br J Pharmacol 130: 1211-1218.

47. Maryon EB, Saari B, Anderson P (1998) Muscle-specific action of ryanodine receptor channel in Caenorhabditis elegans. J Cell Sci 19: 2885-2895.

48. Wahid FN, Behnke JM, Conway DJ (1989) Factors affecting the efficacy of ivermectin against Heligmosomoides polygyrus (Nematospiroides dubius) in mice. Vet Parasitol 32: 325-340.