Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 2, 2015

Original Article

IN VITRO ANTHELMINTIC EFFICACY OF EXTRACTS OF *CITRUS AURANTIFOLIA* (CHRISTM) SWINGLE FRUIT PEELS AGAINST *HELIGMOSOMOIDES BAKERI* OVA AND LARVAE

ONYILOFE SUNDAY ENEJOH[1], MUSA MOHAMMED SULEIMAN[1], JOSEPH OLOGUNJA AJANUSI[2], SULEIMAN FOLORUNSHO AMBALI[1]

[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu BelloUniversity, Zaria, Nigeria
Email: ensunking@gmail.com

Received: 26 Feb 2015, Revised and Accepted: 29 Mar 2015

ABSTRACT

Objective: *In vitro* ovicidal and larvicidal activities of extracts of *Citrus aurantifolia* fruit were investigated on the eggs and first stage larvae of Heligmosomoides bakeri to determine the anthelmintic efficacy of the plant.

Methods: The fruit peels of *Citrus aurantifolia* were extracted with methanol. The crude methanol extract (CME) was dissolved in water and serially partitioned with hexane and ethylacetate to give hexane extract (HE), ethylacetate extract (EE) and aqueous methanol extract (AME). Different concentrations (0.625, 1.25, 2.5, 5, 10, and 20 mg/ml) of the extracts were prepared by dissolving the extracts in distilled water. Two hundred microliters of each concentration was incubated with the larvae and eggs of H. bakeri contained in 0.2 ml solution in a 96-well microtitre plates and incubated at room temperature for 24 and 48 h, respectively. Distilled water and albendazole were used as non-treated and treated controls, respectively.

Results: At 20 mg/ml, the CME, EE, HE and AME inhibited the hatching of H. bakeri eggs by 100±0.0, 98.2±2.7, 97.8±0.68 and 97.7±2.8 %, respectively. Similarly, CME, AME, EE and HE caused death of the larvae of H. bakeri by 100±0.0, 90.8±4.1, 85.9±3.5 and 83.3±6.0 %, respectively. All the extracts showed significant (***p<0.001) anthelmintic effect when compared with the non-treated (DW) control.

Conclusion: This study demonstrates that *Citrus aurantifolia* fruit peels possess anthelmintic activity that might be caused by one or more of the secondary metabolites contained in the plant.

Keywords: *Citrus aurantifolia*, Anthelmintics, Plant extracts, Heligmosomoides bakeri

INTRODUCTION

Helminthosis is an infection of man and animals with parasitic helminthes. The disease is debilitating and chronic in nature in humans. In livestock production, the disease causes clinical and subclinical infections that reduce animal survival, depress growth rate, impair reproductive performance and finally lead to the death of the animals which in turn affect the economic life of humans especially livestock farmers [1-3].

Chemotherapy still remains the most effective way employed in the treatment of helminthosis in the world. However, the high cost of modern anthelmintics coupled with the development of resistance of most helminth parasites to the available anthelmintics has limited the usefulness of these drugs [4]. Other limiting factors to the currently used anthelmintics include: unavailability to rural dwellers and problems of drug residues in animals intended for human consumption, toxicity and slow development of new drugs due to high cost of developing and licensing a new drug [5]. This has led to increasing use of medicinal herbs by Africans and small holder livestock producers [6].

Citrus aurantifolia (Christm) Swingle belonging to the family Rutaceae is one of the most widely used plants especially in African and Asian traditional medicine. The plant is used to treat several diseases such as gastrointestinal disorders, fever, scabies, cough, constipation and malaria [7]. The plant has been shown to possess antioxidant [8], laxative, anti-inflammatory, antihypertensive [9], anticancer [10], antiviral, antimycobacteria [11], antifungi [12] and antibacterial properties [13]. In addition, *C. aurantifolia* fruits are cheap and readily available in many countries of the world. The fruit peels are often removed and discarded as waste products especially in food processing industries. These fruit peels are also rarely consumed by ruminants making the plant a less competitive source of cheap and readily available drug. There is always an increased attention in bringing useful products from waste materials and citrus wastes are no exceptions. Thus, the results of this research can provide alternative anthelmintics from industrial waste products.

Heligmosomoides bakeri (*H. bakeri*) is a trichostrongyliod that occurs naturally in wild mouse populations and has clearly evolved a high level of adaptation to the murine immune system. This worm has been used extensively as laboratory model in screening for anthelmintic drugs [14]. It has a direct life cycle which has many similarities to those of the economically important trichostrongyloid nematodes of sheep, cattle and humans [15]. The aim of this work was to evaluate the *in vitro* anthelmintic efficacy of the fruit peels of *C. aurantifolia* against the ova and first stage larva of *Heligmosomoides bakeri*.

MATERIALS AND METHODS

Plant collection, identification and preparation

Samples of *C. aurantifolia* fruits were obtained from some backyard *C. aurantifolia* trees in Adum-ochi, Ankpa Local Government Area of Kogi State, Nigeria in the first quarter of the year. The plant material was identified at the Herbarium, Department of the Biological Sciences, Ahmadu Bello University, Zaria by a taxonomist, Mr. U. S. Gallah. A sample of the plant was deposited and given a voucher specimen number of 990. The fruits were washed, manually peeled and dried under shade to prevent heat destruction. The dried fruit peels were pounded to powder using wooden mortar and pestle.

Plant extraction and partitioning

The dried pulverized *C. aurantifolia* fruit peels were weighed and extracted by cold extraction (maceration) technique according to method described by Handa *et al.* [16]. About 821 g of the pulverized peel was soaked in methanol in the ratio of 1:3 w/v in a seperatory funnel, whose neck was stucked with cotton wool to allow for filteration of the extract. The set up was allowed to stand for 48 hours and the extract was drained into clean bottles. The procedure was repeated twice using the plant materials and solvent in the ratio

of 1:2 w/v. The liquid methanol extract obtained were pooled together and concentrated to dryness over water baths at 50-60 °C. The brown, semi-solid, oily crude methanol extract obtained was dissolved in distilled water (1:10 w/v) to form an aqueous methanol extract (AME) and serially partitioned with hexane and ethylacetate as solvents. The different portions were concentrated to dryness as described earlier. The portions were subsequently referred to as crude methanol extract (CME), ethylacetate extract (EE), hexane extract (HE) and aqueous methanol extract (AME) and were tested for anthelmintic activities.

Phytochemical test

The extracts were subjected to phytochemical tests using standard techniques described by Trease and Evans [17] to detect phytochemicals in the extracts.

Recovery of *H. bakeri* eggs

Three grams of freshly passed out faeces from artificially infected mice (*Mus musculus)* was collected using a tea spoon into a centrifuge tube and homogenized using a pestle in 12 ml of saturated sodium chloride solution. The solution was filtered through a tea sieve and the filtrate centrifuged at 5 *g* for 5 minutes. The supernatant was decanted into a beaker and 100 ml of distilled water was added to it. The mixture was further centrifuged at 5 *g* for 5 minutes. Using a 10 ml syringe, the supernatant was aspirated and discarded and the sediment re-centrifuged for 5 minutes at 5 *g* after adding 90 ml of water. The sediment obtained after aspirating the supernatant with a syringe was examined under the light microscope at ×10 objective for the presence of *H. bakeri* eggs. The recovered eggs were used for the *in vitro* egg hatch test or cultured at 20 °C for 48 hours to obtain the first stage larvae of *H. bakeri* that was used for larvicidal test.

Evaluation of ovicidal and larvicidal activity of the extracts

The *in vitro* anthelmintic studies involved the evaluation of ovicidal (egg hatch inhibition test) and larvicidal activities of the extracts on the eggs and first stage larvae of *H. bakeri*. Different concentrations (0.625, 1.25, 2.5, 5, 10, and 20 mg/ml) of the extracts were prepared by dissolving the extracts in distilled water. Tween 80 was used to aid the dissolution of EE and HE in distilled water. Two hundred microlitres of each concentration was incubated with the larvae and eggs of *H. bakeri* contained in 0.2 ml solution in a 96-well microtitre plates and incubated at room temperature for 24 and 48 hours, respectively. At the end of 48 hours, a drop of iodine was added to each well containing the eggs to stop further hatching. Albendazole (0.2 ml in each well) of different concentrations (2, 1, 0.5, 0.25, 0.125, 0.0625 mg/ml) and distilled water (0.2 ml in each well) were used as treated and untreated controls, respectively. The plates were covered with foil papers to prevent evaporation.

For the ovicidal test, the content of each well of the microtitre plate was pipetted and placed on a glass slide and examined microscopically at ×10 magnification. All the unhatched eggs as well as the L_1 in each well were counted and recorded.

The percentage inhibition of egg hatching was calculated using the formula described by Wabo *et al.* [18]:

$$\text{Hatching rate (\%)} = \frac{\text{Number of } L_1 \text{ larvae} \times 100}{\text{Number of eggs cultured}}$$

For the larvicidal test, the content of each well was stirred and pipetted onto a clean glass slide and then examined at ×4 magnification. The number of live and dead larvae were counted and recorded. A larva was considered alive if it moved any part of its body or migrated from one point to another; but if the larva showed no observable motion after 10-20 seconds interval, it was considered dead.

The percent mortality (Mc %) was determined using Abott's formula for corrected mortality [19].

$$\text{Mc (\%)} = \frac{\text{Mce} - \text{Mt} \times 100}{100 - \text{Mt}}$$

Where Mce is the mortality obtained during the test and Mt the mortality registered in the untreated control.

Data analysis

Results obtained were expressed as mean±SEM. Analysis of variance (ANOVA) using GraphPad Prism Version 5.0 was used to compare the anthelmintic effects of the different extracts of *C. aurantifolia* fruit peels to albendazole and the non-treated (distilled water) group. The mean in different group was compared using Tukey Post hoc test. Value of $P<0.05$ was considered significant. The 50 % inhibitory concentration (IC50) and the 50 % larvicidal concentration (LC50) were also determined from a log concentration-response curve.

RESULTS

Extract yield

The yield and colour of the crude extract and different portions obtained from the pulverized *C. aurantifolia* are presented on table 1.

Table 1: Yield and colour of extracts of *C. aurantifolia* fruit peels extract after solvent partitioning

	Yield (grams)	Percentage yield	Colour
Crude methanol	110	13*	Brown
Ethylacetate	4.7	6.7	Brown
Butanol	17.5	25	Brown
Aqueous methanol	30.7	43.9	Light brown

*Percentage yield of crude methanol extract from the pulverized *C. aurantifolia* fruit peels

Phytochemical screening

The CME contains carbohydrates, glycosides, triterpenes, tannins, steroids, saponins, sugars, phenols, alkaloids and flavonoids. All the metabolites were present in HE except saponins, phenols and flavonoids. Similarly, sugar and flavonoids were absent in EE. AME also contains all the mentioned metabolites except phenols and alkaloids.

Egg hatch inhibition

The extracts of *C. aurantifolia* fruit peels significantly (*$p<0.05$) inhibited the hatching of eggs of *H. bakeri* in a concentration-dependent manner (fig. 1). The IC_{50} for the CME, EE, AME and HE were 2.74, 3.39, 4.12 and 4.17 mg/ml, respectively.

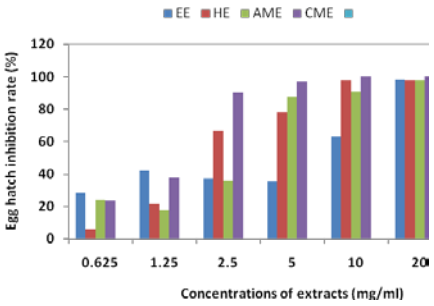

Fig. 1: Inhibitory effects of different concentrations (0.625–20 mg/ml) of crude methanol extract (CME), aqueous methanol extract (AME), ethylacetate extract (EE), hexane extract (HE) of *C. aurantifolia* peels on the egg hatching of *H. bakeri* after 48 hours of incubation

Larvicidal activity of *C. aurantifolia* fruit peel extracts

All the extracts of *C. aurantifolia* fruit peels had significant (**$p<0.01$) larvicidal activity against L_1 of *H. bakeri*. The effect is concentration-dependent (fig. 2). At the concentrations of 5-20 mg/ml, the larvicidal effects produced by all the extracts were not statistically different from one another. The LC_{50} were 0.024, 0.32, 0.99 and 2.15 for AME, CME, EE and HE, respectively.

The ovicidal and larvicidal activities of albendazole are shown on fig. 3. The anthelmintic effect was significant (***P<0.001) with IC_{50} and LC_{50} of 0.96 and 0.00084 mg/ml, respectively.

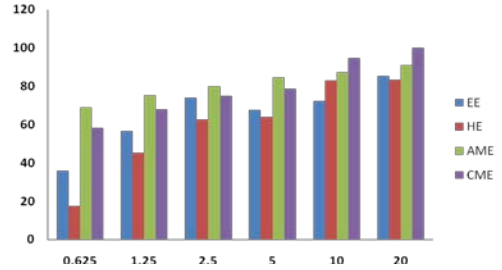

Fig. 2: Effects of different concentrations (0.625–20 mg/ml) of the crude methanol extract (CME), aqueous methanol extract (AME), ethylacetate extract (EE), hexane extract (HE) of *C. aurantifolia* peels on the first stage (L_1) Larvae of *H. bakeri* after 24 hours of incubation at 25˚C

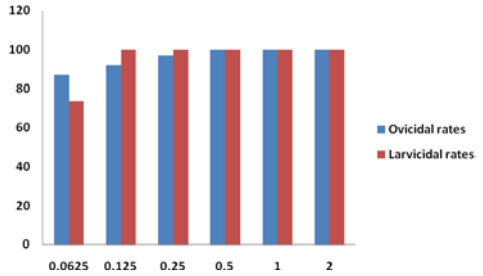

Fig. 3: Ovicidal and larvicidal effects of different concentrations of albendazole on the eggs and first stage larvae of *H. bakeri*

DISCUSSION

In vitro anthelmintic strudies of plants provide preliminary investigations into the anthelmintic properties of the plant. The main advantages of using *in vitro* assays to test for the anti-parasitic properties of plant extracts are that the study is done at a low cost and there are rapid turnover which allows large scale screening of plants. Most anthelmintic activities of plants reported so far were based on *in vitro* studies [20]. The results of this study revealed that CME, AME, EE and HE significantly inhibited the hatching of *H. bakeri* eggs and killed the larvae of the helminth. Even though there were differences in activity between the extracts of the plant, they are not statiscally significant (*p>0.05), especially at the concentrations of 5–20 mg/ml. The effect of CME and AME on egg hatching of *H. bakeri* eggs was not significantly (p>0.05) different from the effect produced by albendazole (standard drug). Studies by other workers using different plants had shown that plant extracts usually produce concentration–dependent effects when tested on helminth eggs [21, 22, 12]. Some members of the Citrus (Rutaceae) family have been shown to possess anthelmintic properties [23, 24]. The egg hatch inhibitory activities of these extracts were more effective with the crude methanol extract. The higher effect produced by crude methanol extract may be due to the synergistic effects of the different chemical constituents of the extract that interacted in complex ways to produce an effect higher than the individual components [25]. The ovicidal effect produced by these extracts might be caused by the penetration of the active phytochemicals into the helminth's egg shell which stopped the segmentation of the blastomere, thus, inhibiting the hatching of the eggs [22].

This study also showed an increase in the mean larval mortality rates with increase in concentration of all the extracts tested. In general, the extract with higher concentrations showed more activity when compared to extract with lower concentration. An increase in concentration represents a supplementary input of different active compound [26]. The extracts showed a highly significant (***p<0.001) larvae mortality rates at the concentration range of 2.5-20 mg/ml. The larvicidal effect of CME and AME on *H. bakeri* larvae was not significantly (p>0.05) different from effect produced by albendazole. Significant difference (**p<0.05) however existed when the larvicidal effect of EE and HE were compared to albendazole. The larvicidal effect produced by these extracts was due to the penetration of the active chemical constituents of the extract across the cuticle of the larvae into their circulatory system when the larvae were brought in contact with the extracts [27].

In this study, the anthelmintic effect of *C. aurantifolia* fruit peels could be attributed to one or more of the phytochemicals present in the plants [28]. The quantitative phytochemical screening of *C. aurantifolia* fruit peel extracts showed that the plant contained carbohydrate, glycosides, steroids, flavonoids, phenol, alkaloids and condensed tannins. However, alkaloids were absent in the hexane and aqueous methanol extracts. Saponins and triterpenes were present in all the portions except in the hexane extract portion. The finding of this study agrees with what was reported earlier by other workers [8, 12, 13, 24, 27].

Previous anthelmintic trials of medicinal plants have shown that the presence of secondary metabolites like alkaloids, saponin, polyphenols, carotenoids, tannins, coumarine, cardenolides, triterpenes, saponoside, embeline, sesquiterpenlactones may be responsible for the plants anthelmintic effect [30, 31]. The tannins contained in plants have been reported to possess anthelmintic activities [32]. The anthelmintic effects of tannins may be attributed to its ability to bind free protein available for larval nutrition and thus reduced nutrient availability which could result in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death. Tannins can also bind to free proteins in the gastrointestinal tract of host animal [31] or glycoprotein on the cuticle of the parasite and cause death [31]. The anthelmintic effect of tannins is similar to some synthetic phenolic anthelmintics like niclosamide, oxyclozanide, bithionol, nitroxynil, etc; which are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [33]. More so, tannins or their metabolites have been shown to have a direct effect on the viability of the preparasitic stages of helminths [34]. The fact that tannins were present in all the extracts of the plant, perhaps, that is why all the extracts showed anthelmintic activities. Saponin contained in plants has been shown to be responsible for the anthelmintic activities of some medicinal plants. Saponins act by destabilizing the membranes and increasing the cell permeability in helminth parasites [35]. Limonene was believed to be the active principle in *citrus* fruit peels that caused its anthelmintic activity in ruminants [36]. The mode of action of limonene is unknown; however investigations suggested that it has inhibitory effects on nematode growth and also interfere with parasite enzymes [36].

In this study, there was variation in the level of larvicidal and ovicidal effects shown by the extracts. The extract showed more larvicidal effect. This difference could be attributed to the egg shell resistance. The egg shell comprises three layers; an external lipoprotein layer, a middle chitinous protein layer and an inner lipid layer. The middle and inner layers are resistant to salts and chemicals and also protects the eggs from desiccation, strong acid and bases, oxidants, reductive agents, detergents and proteolytic compounds [37]. This egg shell resistance limited the penetration of the extracts into the egg and thus, lowered the effects the extracts had on the egg when compared to that of the larvae.

Albendazole showed a higher anthelmintic activity than *C. aurantifolia* fruit peels extracts in this study even though the concentrations of albendazole were ten times lower. This superior anthelmintic activity of albendazole could perhaps be attributed to its purity when compared with the extracts which may have other compounds contained in it. Albendazole is a broad-spectrum anthelmintics; and it has been shown to possess both ovicidal and larvicidal activities [38].

CONCLUSION

From the above results, we concluded that the extracts of *C. aurantifolia* fruit peels proved to have ovicidal and larvicidal

properties. They were able to inhibit the egg hatching as well as larval survival of *H. bakeri.* Further studies incorporating *in vivo* studies are required to find out and establish the effectiveness and pharmacological rationale for the use of *C. aurantifolia* fruit peel extracts as anthelminthic drugs.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGMENT

The authors are grateful to the technical staff of the Departments of Veterinary Pharmacology and Toxicology, Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for their technical support. Dr. L. A. Ngongeh is thanked for providing the helminth parasite used in this study.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Perry BD, Randolph TF. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet Parasitol 1999;84:145-68.
2. Waller PJ. Nematode parasite control of livestock in the tropics/subtropics: the need for novel approaches. Int J Parasitol 1997;27:1193-201.
3. Githiori JB, Hoglund J, Waller PJ, Baker RL. Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. Parasitol 2004;129:245–53.
4. Saeed M, Iqbal Z, Jabbar A. Oxfendazole resistance in gastrointestinal nematodes of beetal goats at livestock farms of Punjab (Pakistan). Acta Vet Brno 2007;76:79–85.
5. Gasbarre CL, Stout LW, Leighton AE. Gastrointestinal nematodes of cattle in the northeastern US: results of a producer survey. Vet Parasitol 2001;101:29–44.
6. Kudi AC, Myint SH. Antiviral activity of some Nigerian medicinal plant extracts. J Ethnopham 1999;68:289-94.
7. Abo KA, Fred-Jaiyessimi AA, Jaiyesimi AEA. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. J Ethnopham 2008;115:67-71.
8. Reddy JL, Reshma DH, Beena J, Spandana G. Evaluation of antibacterial and antioxidant activities of the leaf essential oil and leaf extracts of *Citrus aurantifolia*. Asian J Biochem Pharm Res 2012;2(2):2249–4626.
9. Souza A, Lamidi M, Ibrahim B, Aworet SRR, Boukandou M, M'Batchi B. Antihypertensive effect of an aqueous extract of *Citrus aurantifolia* (Rutaceae) (Christm.) Swingle, on the arterial blood pressure of mammal. Int Res Pharm Pharm 2011;1(7):142-8.
10. Jaiprakash RP, Jayaprakasha GK, Mahadev BC, Bhimanagouda SP. Bioactive compounds from maxican lime (*Citrus aurantifolia*) juice induce apoptosis in human pancreatic cells. J Agric Food Chem 2009;57(22):10933-42.
11. Nallely E, Sandoval M, Abraham G, Elizondo-Treviño E, Garza-González E, Alvarez, *et al.* Chemical composition of hexane extract of *Citrus aurantifolia* and anti-mycobacterium tuberculosis activity of some of its constituents. Molecules 2012;17:11173-84.
12. Dongmo PMD, Tatsadjieu LN, Tchinda ES, Kuate J, Amvam PHZ, Menut C. Essential oils of *Citrus aurantifolia* from Cameroon and their antifungal activity against *Phaeoramularia angolensis.* Afr J Agric Res 2009;4(4):354-8.
13. Loizzo MR, Tundis R, Bonesi M, Menichini F, De Luca D, Colica C, *et al*. Evaluation of *Citrus aurantifolia* peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. J Sci Food Agric 2012;24(12):18930-7.
14. Wabo PJ, Payne VK, Mbogning TG, Komtangi MC, Yondo J, Ngangout MA, *et al*. *In vitro* anthelminthic efficacy of *Dichrocephala integrifolia* (Asteraceae) extracts on the gastro-intestinal nematode parasite of mice: *Heligmosomoides bakeri* (*Nematoda, Heligmosomatidae*). Asian Pac J Trop Biomed 2013;3(2):100-4.
15. Behnke JM, Menge DM, Noyes H. *Heligmosomoides bakeri*: a model for exploring the biology and genetics of resistance to chronic gastrointestinal nematode infections. Parasitol 2009;136(12):1565-80.
16. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. Int centre for science and high technology, Trieste; 2008. p. 21-5.
17. Trease and Evans, *Pharmacognosy*. Springer publication; 2007. p. 153-7.
18. Wabo Poné J, Bilong BCF, Mpoame M. *In vitro* nematicidal activity of extracts of *Canthium mannii* (Rubiaceae) on different life cycle stages of *Heligmosomoides polygyrus* (*Nematoda, Heligmosomatidae*). J Helmenthol 2010;84:156-65.
19. Wabo Poné J, Mpoame M, Bilong BCF, Kerboeuf D. *In vitro* comparative study of the nematicidal activity of ethanolic extract of the powder of the stem bark of *Canthium mannii* (Rubiaceae) and the Me^2bendazole. Revue Méd Vét 2005;156(12):633-6365.
20. Mali RG, Mehta AA. A review of Anthelmintic plants. Nat Prod Rad 2007;7(5):466-75.
21. Ademola O, Eloff JN. Anthelmintic efficacy of cashew (*Anarcadium occidentale* L.) on *in vitro* susceptibility of the ova and larvae of *Haemonchus contortus.* Afr J Biotech 2011;10(47):9700-5.
22. Kollins EN, Wabo PJ, Payne VK, Jeannette Y, Marie-Claire K. *In vitro* comparative effect of aqueous (Cold and Hot Water) and ethanolic extracts of the seeds of *Aframomum Danielli* (Zingiberaceae) on three life cycle stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda; Heligmosomatidae), Parasite of the Laboratory Mice (*Mus Musculus*). Med Aromat Plants 2012;1:11.
23. Rajarajan AT, Vijayasree VG, Kenichi W, Vijaya S, Narasimman G, Sadish SK. Anthelmintic and antimicrobial properties of fruit peels of *Citrus sinensis*. Pharmacologyonline 2009;1:363-8.
24. Bairagi GB, Kabra AO, Mandade RA. Anthelmintic activity of *Citrus limon* L. (burm) fruit peels extract in Indian adult earthworm. Int J Pharm Tech 2011;3(2):664-7.
25. Cock IE. Problems of Reproducibility and Efficacy of bioassays using crude extracts, with reference to *Aloe vera.* Pharmacogn Commun 2011;1(1):52-62.
26. Wabo PJ, Yondo J, Fossi TO, Marie CK, Bilong BCF, Mpoame M. The *in vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri* (*Nematoda, Heligmosomatidae*). J Pharmacogn Phytochem 2011;3(4):56-62.
27. Payne VK, Kollins EN, Wabo PJ, Yondo J, Komtangi M, Mpoame M, *et al. In vitro* comparative effect of extracts of the seeds of *Embelia rowlandii* (Myrsinaceae) on the eggs and L1 and L2 larval stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda; Heligmosomatidae). Afr J Biotech 2013;12(2):205-11.
28. Abdelqader A, Bassam Q, Diya A, Gürbüz D. Anthelmintic effects of citrus fruit peels ethanolic extracts against *Ascaridia galli.* Vet Parasitol 2012:1-7.
29. Pathan RK, Papi RG, Parveen P, Tananki G, Soujanya P. *In vitro* antimicrobial activity of *Citrus aurantifolia* and its phytochemical screening. Asian Pac J Trop Dis 2012;2012:328-31.
30. Suleiman MM, Mamman M, Aliu YO, Ajanusi JO. Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. Veterinarski Arhiv 2005;75:487-95.
31. Athanasiadou S, Githiori J, Kyriazakis I. Medicinal plants for helminth parasite control: facts and fiction. Animal 2007;1:1392–400.
32. Ngongeh LA, Fakae BB. Effect of quebracho tannin drenching on *Heligmosomoides bakeri* infection in mice. Int J Sci Nat 2011;2(1):132-5.
33. Bate-Smith EC. The phenolic constituents of plants and their taxonomic significance. J Linn Soc London Bot 1962;58:95-173.
34. Duncan RA. An evaluation of suitable bioassays to measure the potential of condensed tannins to inhibit the viability of sheep nematode parasites. Bachelor of Rural Science, Honours thesis,

Animal Science, University of New England, Armidale, NSW, Australia; 1996. p. 34.

35. Price KR, Johnson INT, Fenwick GR. The chemistry and biological significance of saponin in food and feed stuffs. Res Food Sci 1987;26:27-135.
36. Squires JM, Foster J, Lindsay D, Caudell D, Zajac A. Efficacy of an orange oil emulsion as an anthelmintic against *Haemonchus contortus* in gerbils (*Meriones unguiculatus*) and in sheep. Vet Parasitol 2010;172:95–9.
37. Jimenez-Cisneros BE. Helminth ova control in waste water and sludge for Agricultural reuse. In: Grabow WOK, Editor. Encyclopedia of Life Support Systems (EOLSS); 2007. p. 3-9.
38. Riviere RE, Papich MG. Veterinary Pharmacology and Therapeutics. 9th ed. Wiley-Black; 2009. p. 1053-145.