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Evaluation of Ovicidal and Larvicidal Activities of Methylene Chloride-Methanol Extract of *Annona Senegalensis* (Annonaceae) Stem Bark on *Heligmosomoides Bakeri* (Nematoda, Heligmosomatidae)

By Francesco D'Angelo, Wabo Poné J, Yondo Jeannette, Komtangi Marie Claire, Sauro Vittori & Mpoame Mbida

University of Dschang, Cameroon

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Keywords: *heligmosomoides bakeri; annona senegalensis; ovicidal; larvicidal; anthelminthic.*

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Keywords: heligmosomoides bakeri; Annona senegalensis; ovicidal; larvicidal; anthelminthic.

Author α ¥: School of pharmacy, University of Camerino, 62032 Camerino (MC), Italy.
Author σ ρ ω §: Department of Animal Biology, Laboratory of Applied Biology and Ecology (LABEA), Faculty of Science, University of Dschang, PO Box 067 Dschang, Cameroon

I. Introduction

Infections of animals with gastrointestinal nematodes constitute a world wide health problem. These parasites frequently cause death in heavily infected host, resulting in important economic losses. Chronic infections are dangerous and can cause a reduction of milk and meat production, fertility and growth in animals. The anthelminthics discovered in the '60s, and which were largely used in the last 50 years, limited the problem until resistance, and multi-resistance, appeared in nematode populations (Holmes, 1985; Zajac et Gipson, 2000). Actually, in certain regions of the world, mostly among the developing countries, the situation is very serious (Van wyk et al. 1999). In fact, in tropical and sub-tropical zones, the spread of gastro-intestinal parasitic infections is strongly increased by climatic factors, such as temperature and humidity, and poor hygiene (Satrija et al. 1995). In Cameroon, the Permanent Secretary of the National Programme for the control of Schistosomiasis and Gastrointestinal Helminthiases reported in January 2006 that, over 10 millions natives are infected with various intestinal parasites and that 2 milions suffer from schistosomiasis. These infections affect children of school age especially, influencing stronly their growth, intellectual development and vulnerability to other diseases (Ngangout et al., 2012). The control measures used today depend on vaccination (Newton & Mun, 1999), breeding of sheep resistance races (Gray, 1997), improving food quality (Wallace et al. 1998) and breeding dairy farming methods (Bargers, 1999; Niezen et al. 1999). In addition, some biological measures are being developed such as the optimization for the use of anthelminthics already available on the market (Larsen, 1999; Van Wyk & Bath, 2002).

In this context, the search for new nematocidal agents is still a priority for humans and for livestock (Witty, 1999). Synthetic anthelminthics still dominate the

animal pharmaceutical industry and they are widely sold at high prices (see International Federation for Animal Health website: http://www.ifahsec.org). The massive and strategic use of modern anthelminthic drugs is widely spread to control worm infections. Unfortunately, the high cost of these medicines, the increasing development of resistant strains of gastro-intestinal parasites and the shortage of veterinarians, especially in poor countries, push livestock breeders and herdsmen towards traditional medicine using therapheutic plants (Kaboré et al., 2005).

For a long time, humans have developed throughout the world a traditional pharmacopea based on the knowledge of medicinal plants. This knowledge became highly enriched over time through the observation of animal behaviour and through experimental tests. In many cases, the information thus acquired is only orally transmitted and it is therefore dangerously being put out in favour of modern medicine. In fact, traditional medicine still represents the cheap possible and readily available treatment for the natives. it is also an important potential source for the development of new pharmacological agents. Thus it is clear that modern ethnobotanical research is both important and urgent (Schillhorn Van Veen, 1997; Hammond et al., 1997). various studies, and some of these studies have shown that many plants contain several molecules with antiparasitic activity, such as quinine and artemisin, that are presently widely used (Kayser et al., 2003), while some other plants show promising anthelminthic activity (Al-qarawi et al., 2001; Onyeyilli et al. 2001). However, studies that scientifically evaluate the effectiveness of traditional medicine are very few (Lans & Brown, 1998; Veira et al., 1999), and publications related to Africa are even fewer (Aké Assi & Guinko, 1991; Nfi et al., 2001). Besides, the need for stringent regulation mechanisms and secrecy over the use of traditional medicine remains the major stumbling block (Gqaleni et al., 2007). Furthermore, the inadequate toxicological evidences about the safety of these medicinal plants is still an unsolved problem (Fennel et al., 2004).

The resistance against medically active substances is particularly pronounced in the case of gastrointestinal nematodes of sheep and goats (Sangster, 1999), and especially in the cases of Haemonchus contortus and Heligmosomoides polygyrus (now known as *H. bakeri*) (Diehl et al., 2004; Githiori et al. 2003).

Annona senegalensis (also known as African custard apple), is one of the medicinal plants that are commonly used in Central Africa against gastrointestinal parasite infections. This is a flowering plant of the custard apple family that takes the form of either a shrub or a small tree, growing up to, between two and six meters tall. Its bark has a smooth or a coarse texture, and may be gray-silver or gray-brown. It is leaf-scared, with nearly round flaking, showing lighter-hued spaces under the bark. Branches have thick, gray, brown or yellow tomentum when they are still new. Leaves are from green to blue-green and flowering white creamy. The fruits are made up of numerous fused, fleshy, bumpy, ovoidal or globular carpels. *Annona senegalensis* is origin to east and northeast, west and central, and southern tropical Africa, and to islands in the western Indian Ocean. In South Africa, it is found in KwaZulu-Natal, Limpopo and Mpumalanga (Anonymous, 2010). In vitro studies on aqueous and ethanolic extracts of the bark of stem, of this plant, have already confirmed their ovicidal and larvicidal activity on nematodes (Ngangout et al., 2012). In fact, the bark of this plant was used in early history to prepare tisanes and tea herbs for treating a wide array of ailments such as: intestinal and tissue parasitic worms, diarrhea, gastroenteritis, lung infections, toothaches and even snakebites (Anonymous, 2010). The aim of this study was to assess the in vitro ovicidal and larvicidal activity of Methylene Chloride/Methanol 1:1 volume mixture of extract from bark of the stem of *A. senegalensis* on *H. bakeri* eggs and larvae obtained from the faeces of experimentally infected mice.

II. Materials and Methods

a) Plant materials

The bark of stem of *A. senegalensis* used in this experiment was collected from the peripheral savanas of Foumban, Noun Division, West Region of Cameroon. It was then air dried while protected from sunlight and dust. Finally, the bark was well cut in small pieces (3 cm x 4 cm), ground and kept in the laboratory for further use.

b) Preparation of plant extract

The procedure used was according to Wabo Poné et al. (2006, 2010). Briefly, 200 grams of stored powder were macerated in 1.5 L of methanol (purity > 95%) and 1.5 L of Methylene Chloride (purity > 95%). The Methylene Chloride-methanol mixture was used for its greater capacity to extract some specific compound materials of the plant. The mixture was placed in an airtight glass jar and kept in a dark place. It was stirred daily to accelerate extraction. Seventy two (72) hours later, it was filtered through two metallic sieves (mesh sizes: 500 µm and 150 µm), then through a cotton layer and finally through filter paper of pore size of 2.5 µm. The solution obtained was then concentrated in a rotavapor at ~80 °C for 2 hours (rotation rate: ~150 rounds/min). The extract obtained, was poured in two large glass containers, covered with paper, and kept in the oven at 40-45 °C for 24 hours to allow it for a complete evaporation of the solvents. Two hundred (200) mg of the obtained extract were dissolved in 0,8 mL of Tween 80 (to facilitate the mixture with water). Warm water was finally added to bring the solution to a

volume and prepare 20 mL of stock solution at 10 000 µg/mL. Through a series of dilutions were made to obtain the following concentrations: 7 500, 5 000, 2 500 and 1 250µg/mL.

0.8 mL of Tween 80 were added to 19.2 ml of distilled water to obtain a solution at 4 % V/V, used for negative control.

c) Recovery of nematode eggs

Mice (Mus musculus) were infected by oral gavage with their natural nematode, *Heligmosomoides bakeri* (previously known as Nematospiroides dubius, H. polygyrus and H. p. bakeri).The eggs of *H. bakeri* were obtained from the faeces of mice as follows. Each day, ~100 mg of faeces were collected from the animal cages, homogenized in a mortar adding a small volume of water, suspended in saturated salt solution (NaCl 40 % W/V). Then the solution was cleared of organic debri by filtration through a 250 µm mesh-size sieve into a beaker and finally poured into three glasses of U-vials until the formation of a meniscus at the top. A cover slide was thus deposited on each vial, in direct contact with the solution. Three (3) minutes later, the cover slides were removed and put on a slide and soon analysed under an optical microscope Olympus-CH under the 4x objective to look for eggs. Slides and cover slides containing the eggs were finally rinsed with tap water into a 50 mL vial. The vial was allowed to stand for 30 minutes for the sedimentation of the eggs at the bottom. Then, to completely remove the salt solution, 40 mL of solution were accurately siphoned out using a syringe and replaced with the same amount of tap water. This operation was repeated each 30 minutes three times. Finally the supernatant was removed and the remaining solution was distributed into 24 Petri dishes (Ø = 35 mm) each with 1 mL.

Figs 1 and 2 show *H. bakeri* fresh and embryonated eggs seen through the optical microscope, respectively. Here it is easy to recognize the L1 stage larvae still within the shell of the eggs.

d) Evaluation of ovicidal activity

The ovicidal activity was determined using two different parameters: the effect on the embryonation rate and the hatching rate. To evaluate the effect on the embryonation rate, a percentage of embryonation inhibition (EI) was calculated using the following formula:

$$EI\ \% = 100 - [\left(\frac{Number\ of\ L_1\ larvae}{Number\ of\ fresh\ eggs\ in\ culture}\right) x100]$$

The eggs in 6 Petri dishes were counted immediately after their introduction (20-40 eggs/Petri dish) and soon each, Petri dish received 1 mL from each one five different extract concentrations or 1 mL of Tween 80 solution (4 % V/V) for the negative control. After 48 hours, the larvae in each Petri dish were counted. It is assumed that the embryonated eggs contain the 1st larval stage.

To evaluate the hatching rate, a percentage of hatching inhibition (HI) was calculated by the formula below:

$$HI\ \% = 100 - [\left(\frac{umber\ of\ L_1\ larvae}{Number\ of\ embryonated\ eggs\ in\ culture}\right) x\ 100]$$

In this case, 1 mL of each one of five different concentration extracts or Tween 80 solution (4 % V/V) was introduced into 6 different Petri dishes 24 hours after the collection of eggs, just when these start to hatch. The counting of the larvae was done 6 hours later, when 90 % of eggs are hatched in the negative control.

In all Petri dishes, the final tested concentrations were behalfed from the initial ones due to the addition of egg suspension (1mL) resulting as follows: 5 000, 3 750, 2 500, 1 250 and 625 µg/mL for the plant extract, and 2 % V/V Tween 80 aqueous solution for the negative control. Each treatment was repeated 4 times.

e) Recovery of nematode L1 and L2 larvae

To obtain larvae of the parasite, some eggs, kept inside the vials, were maintained in culture at room temperature (24-25 °C) for fixed periods of time.

L1 larvae were soon identified after hatching from eggs. The larvae were incubated in the extract and tween around 30 hours after the collection of the eggs from faeces. This is when the number of larvae in the negative control reaches 90 % of the number of eggs present in a Petri dish.

To obtain L2 larvae, a solution of faeces collected from the mice free of parasite was added to the egg suspension and kept in a temperature room (24-25 °C) for about 48 hours. The addition of the extract was made only after the identification of L2 larvae. In fact, there are some morphological and specific characteristics that can be exploited, during the qualitative analysis under the microscope, to distinguish between the first and second stage of larvae (L1 larvae are smaller than L2 larvae and do not present the internal esoskeleton that is characteristic of adult larvae. Further more, L1 larvae movements are limited while L2 larvae agitate so fast in "S-shaped" movements like a snake. Figs 3 and 4 show these evident differences between L1 (Fig. 3) and L2 (Fig. 4) larvae.

f) Evaluation of larvicidal activity

To assess the effect of larval extract on the survival, 1 mL of solution containing each 15-25 larvae, previously poured into 6 Petri dishes, was mixed with the same volume of extract at the following final concentration (5 000, 3 750, 2 500, 1 250 and 625 µg/mL). The negative control thus reached at the ratio of

$$MR\ \% = 100x(\frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ in\ culture})$$

Immobile and straight shaped larvae were stimulated with a light-heating ray for about 20-30 seconds to confirm death (Fig. 5). Furthermore, their external layer is not uniform and presents holes and irregularities. In all the cases, the number of dead or immobile larvae was counted 2, 4, 6, and 24 hours after incubation in the extract.

g) Statistical analysis

The inhibitory 50 % concentrations (IC50) for embryionation and hatching rates were calculated using the regressive line of the data obtained from the tests, using the high value of the coefficient of determination (R2). The lethal 50 % concentrations (LC50) for L1 and L2 larvae mortality rates were determined using the regressive line of the probit as a fonction of the natural logarithm of the concentrations (also in this case the coefficient of determination R2 was taken into account). The mean percentages of inhibition and larval mortality were compared using Chi-square test and the differences were considered significant at $P < 0.05$.

III. Results

The yield of the extraction process with the mixture of Methanol and Methylene Chloride (1:1 V/V) was of 8.95 %, thus resulting in 17.9 g of dried extract (starting from 200 grams of dried powder).

The effect of various concentrations of plant extracts on embryonnation rate are shown in Figure 6a. The negative control (Tween 80 aqueous solution at 2 % V/V) hardly affected embryonation rate (4.50% inhibition rate). Embryonation inhibition rate was concentration dependant. The highest concentration of extract (5000 µg/mL), yielding the highest embryonation inhibition rate (20.80%). The calculated IC50 value for the embryionation rate is 15 930 µg/mL (R2 = 0,92).

The percent inhibition of *H. bakeri* eggs hatching, in various concentration of Methylene Chloride-Methanol exctract of *A. senegalensis* barks of the stem, is shown in Figure 6b. In the negative control the mean hatching inhibition rate of the eggs is very low (only the 3 % of eggs affected). Forty eight (48) h post-treatments hatching inhibition rate was clearly concentration dependant (9.00%. at 625 µg/mL to 16.10% at 5 000 µg/mL).The calculated IC50 value for the hatching rate is 23 910 µg/mL (R2 = 0, 97).

2 % V/V of Tween 80 in water. All the treatments were repeated 4 times.

To evaluate larval mortality, the number of dead or immobile larvae was accurately counted under the microscope with the 4x objective. Then, the percentage of mortality rate (MR) was calculated using the following formula:

The effect of *A. senegalensis* extract on the survival of *H. bakeri* L1 larvae is reported in Figure 7a which presents the mean mortality rate obtained two, four, six and twenty four hours post-treatment. The negative control didn't cause any larval mortality. Larval mortality was concentration and time dependant. The highest value (100 %) being recorded in 5 000 µg/mL extract concentration in only 6 hrs. The calculated LC50 values for the L1 larvae mortality rate are as follows: 5 000 µg/mL (R2 = 0.97), 426 µg/mL (R2 = 0.76), 167 µg/mL (R2 = 0.92), 25 µg/mL (R2 = 0.93) for 2, 4, 6 and 24 h, respectively. Figure 7b shows the activity of *A. senegalensis* barks stem extract on *H. bakeri* L2 larvae survival. The absence of activity in the negative control demonstrates the safety of 2 % Tween 80 aqueous solution for *H. bakeri* larvae. The concentration and time dependence recorded for L1 larvae was also observed in this case. The calculated LC50 values for the mortality rate are as follows: 21 320 µg/mL (R2 = 0.92); 2 407 µg/mL (R2 = 0.96); 1 849 µg/mL (R2 = 0.98); 236 µg/mL (R2 = 0.84) after 2, 4, 6 and 24 h respectively.

IV. Discussion

Taking into account the data presented, it is thus easy to understand that the Methylene Chloride-Methanol extract of *A. senegalensis* barks stem, possesses some anthelminthic properties against *H. bakeri* eggs. These findings could be due to the polarity of the solvents used for the extraction and consequentely to the secondary metabolites extracted from the plant material. Thus methanol (a highly polar solvent; dielectric constant 32.6) will extract tannins, catechins, terpenoids, polyphenols and alkaloids while Chloride (apolar solvent; dielectric constant 9.1) will extract semi-polar and apolar compounds, such as heterosides, terpenes, sterols, coumarins and carotenoids (Marie-Magdeleine et al. 2010a). Many studies have revealed the saponins among the most active compounds in terms of nematotoxic activity because of their specific interactions with the cell membranes and with the collagen proteins present on the cuticles of the parasite larvae, causing changes in cell wall permeability and cellular death (Heng et al.,2004; Argentieri et al.. 2008; Eguale & Giday, 2011). In this work, saponins were not present in the *A. senegalensis* Methylene Chloride and Methanol extracts. Our hypothesis is that, the compounds extracted by the two solvents are not capable of passing through the

parasite fresh egg shell, and therefore cannot affect mean embryonation and hatching rates of *H. bakeri* eggs.

On the other hand, the effect of the extracts against L1 and L2 *H. bakeri* larvae is very high. Mortality rate, reaching 100 % in some cases, as described in the previous session. In each assay, the data obtained in the negative controls demonstrates that the used excipient (Tween 80 2 % V/V) can be considered totally ineffective and thus any activity observed in assays can be completely ascribed to plant extracts. The results show that *A. senegalensis* Methylene Chloride/Methanol extracts are much more active on L1 larvae than on L2 ones. These findings could be related to the presence of flavonoids and alkaloids, known to possess strong larvicidal properties. In fact, the activity of flavonoids is attributed to their anti-oxidative and free-radical scavenging capacity (Middleton et al., 2000). As for alkaloids, they creat alkaline conditions which are deleterious to the survival of parasitic larvae that normally prefer acidic conditions (Wabo Poné et al. 2011). Thus the larvicidal activity observed, may be related to two different mechanisms of action, the first, creating unsuitable environmental conditions for the survival of larvae and the second, affecting the internal organs of the larvae either through. interference with the neuromuscular physiology or blockage of energy metabolism (Nchu et al., 2011). To this purpose, it is necessary that the active compounds are found within the larval body and spread in the intestinal cells (Marie-Magdeleine et al. 2010b). The active molecules may either enter the larvae through the cuticle or through ingestion, during feeding. It is possible that the Methylene Chloride-Methanol extract of *A. senegalensis* could contain a large spectrum of active compounds, characterized by different modes of actions. In fact, the bark of plant stems represents the site of the biosynthesis and storage of secondary metabolites that probably are responsible for the biological properties of medicinal plant extracts.

The Methylene Chloride/Methanol extract of *A. senegalensis*, compared to the aqueous and ethanolic extracts of the same plant,[6] show a lower effect on the eggs of *H. bakeri* but, at the same time, a surprisingly higher activity on the parasite larvae.

In conclusion, the observations brought out in this work, reveal that the Methylene Chloride/Methanol extract of *A. senegalensis* shows an ovicidal and larvicidal activity. The activity of the plant, very high against *H. bakeri* L1 and L2 larvae, is probably related to the combined interaction of the extracted compounds as described above. However, in vivo further studies are needed to confirm its efficacy and mostly, to investigate the potential presence of toxic effects.

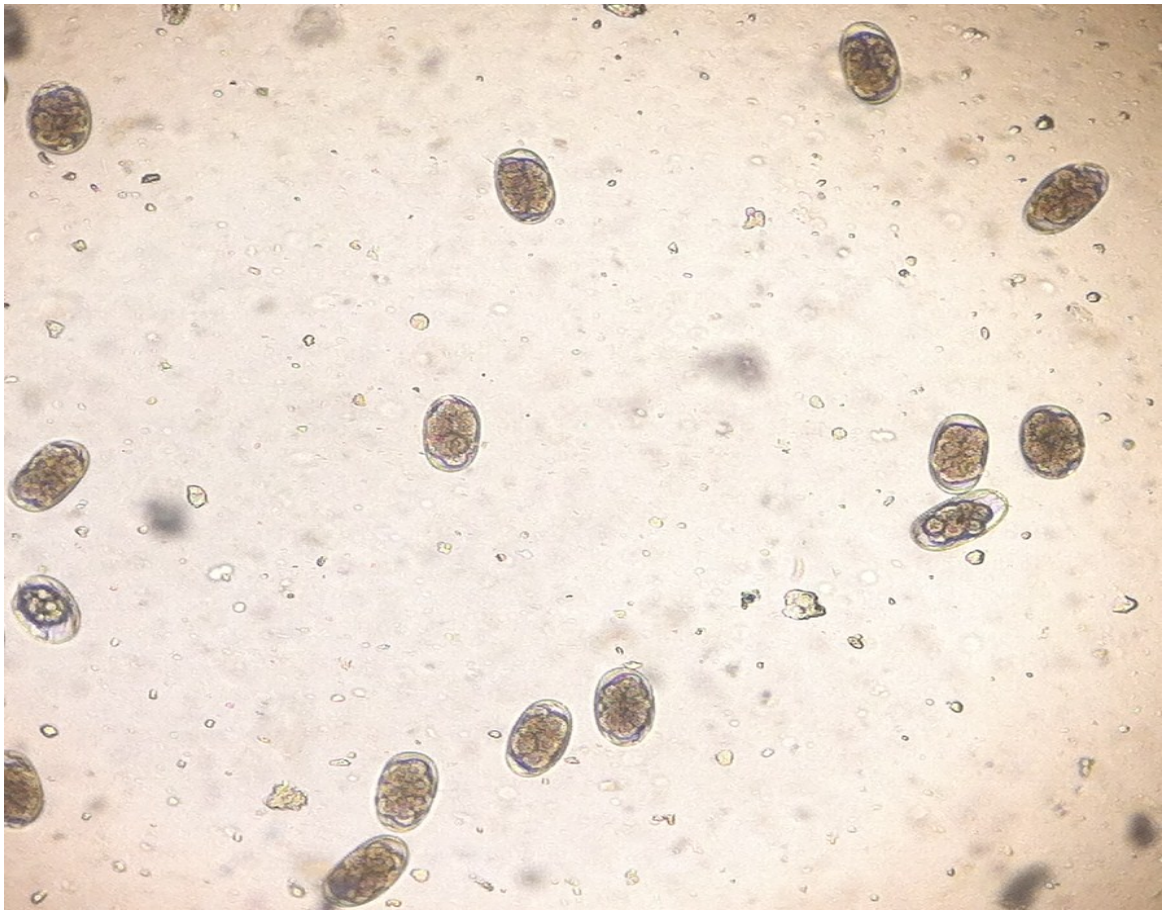

Figure 1 : Fresh eggs of *Heligmosomoides bakeri* (x100)

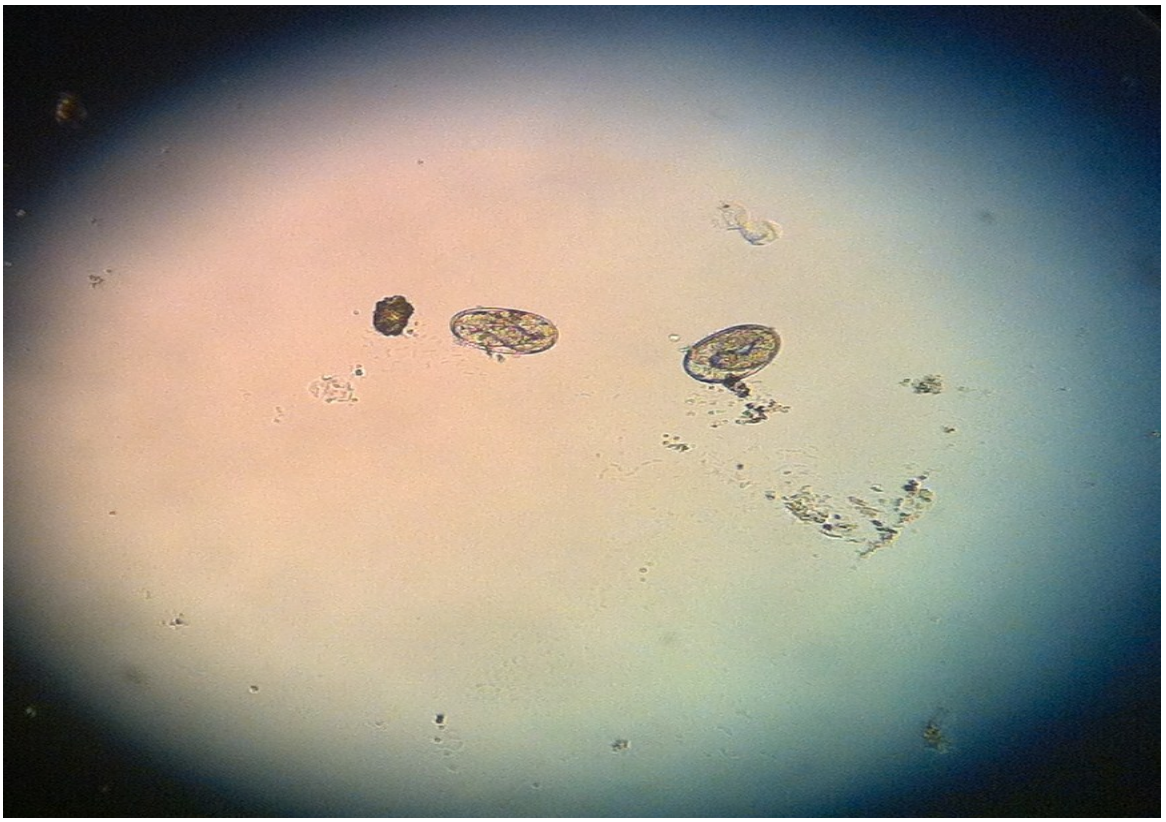

Figure 2 : Embryonated eggs of *Heligmosomoides bakeri* (24 hrs-old). X 100

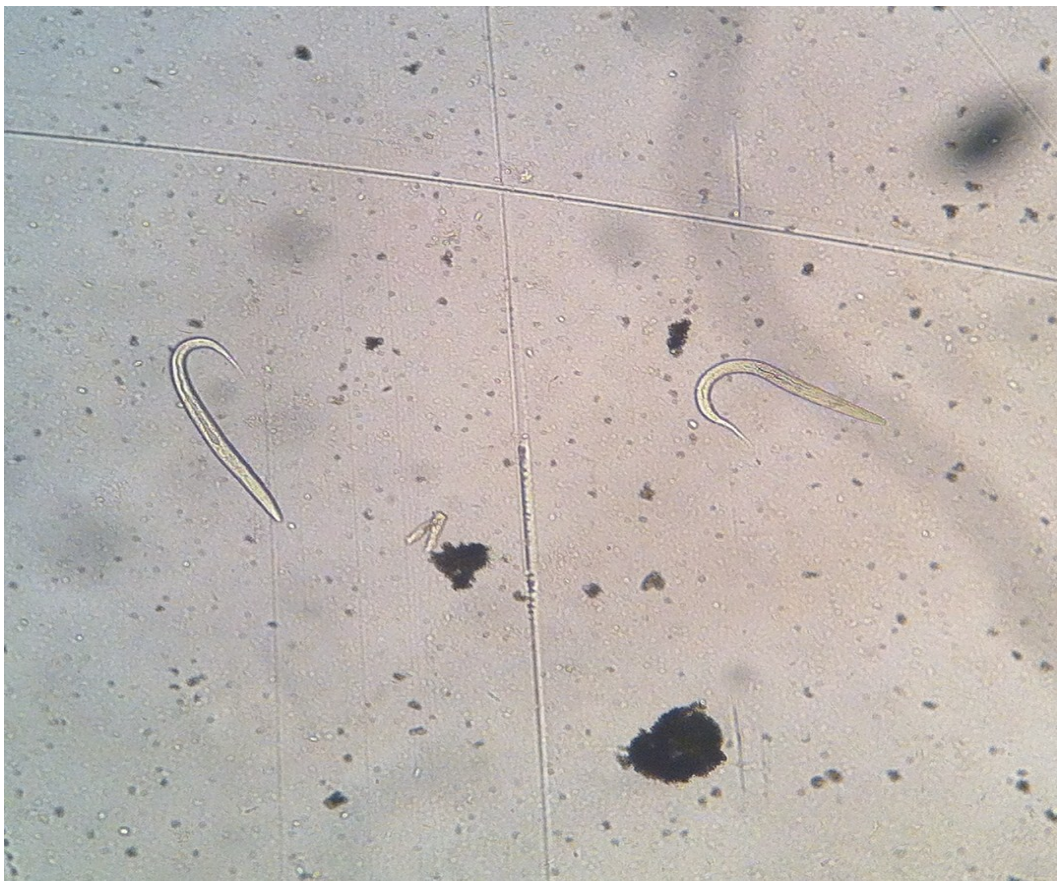

Figure 3 : L1 larvae of *Heligmosomoides bakeri* (x100)

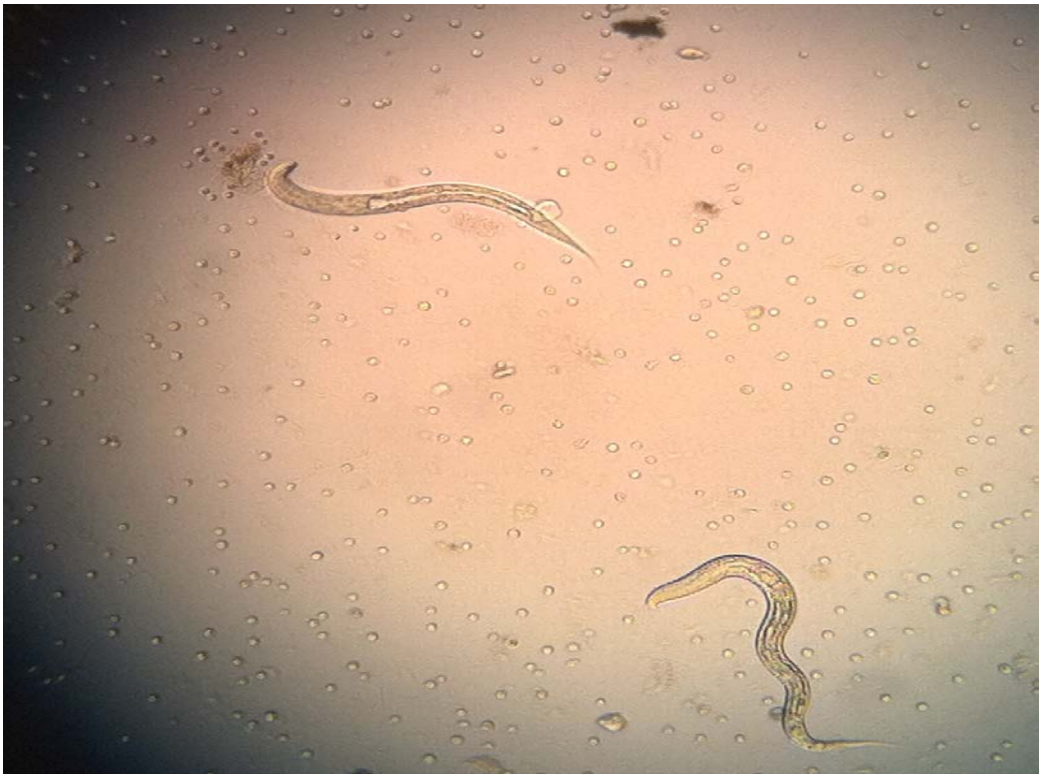

Figure 4 : L2 larvae of *Heligmosomoides bakeri* (x 100)

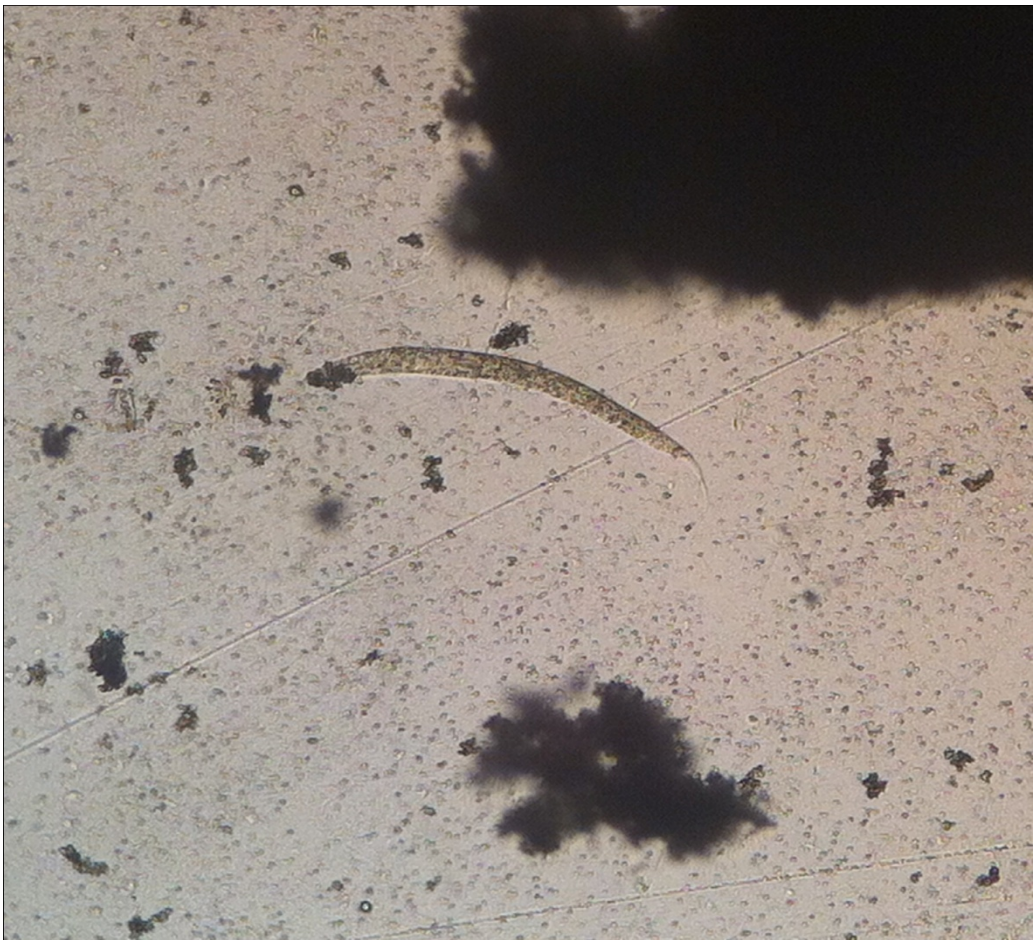

Figure 5 : *Heligmosomoides bakeri* dead larva (x100)

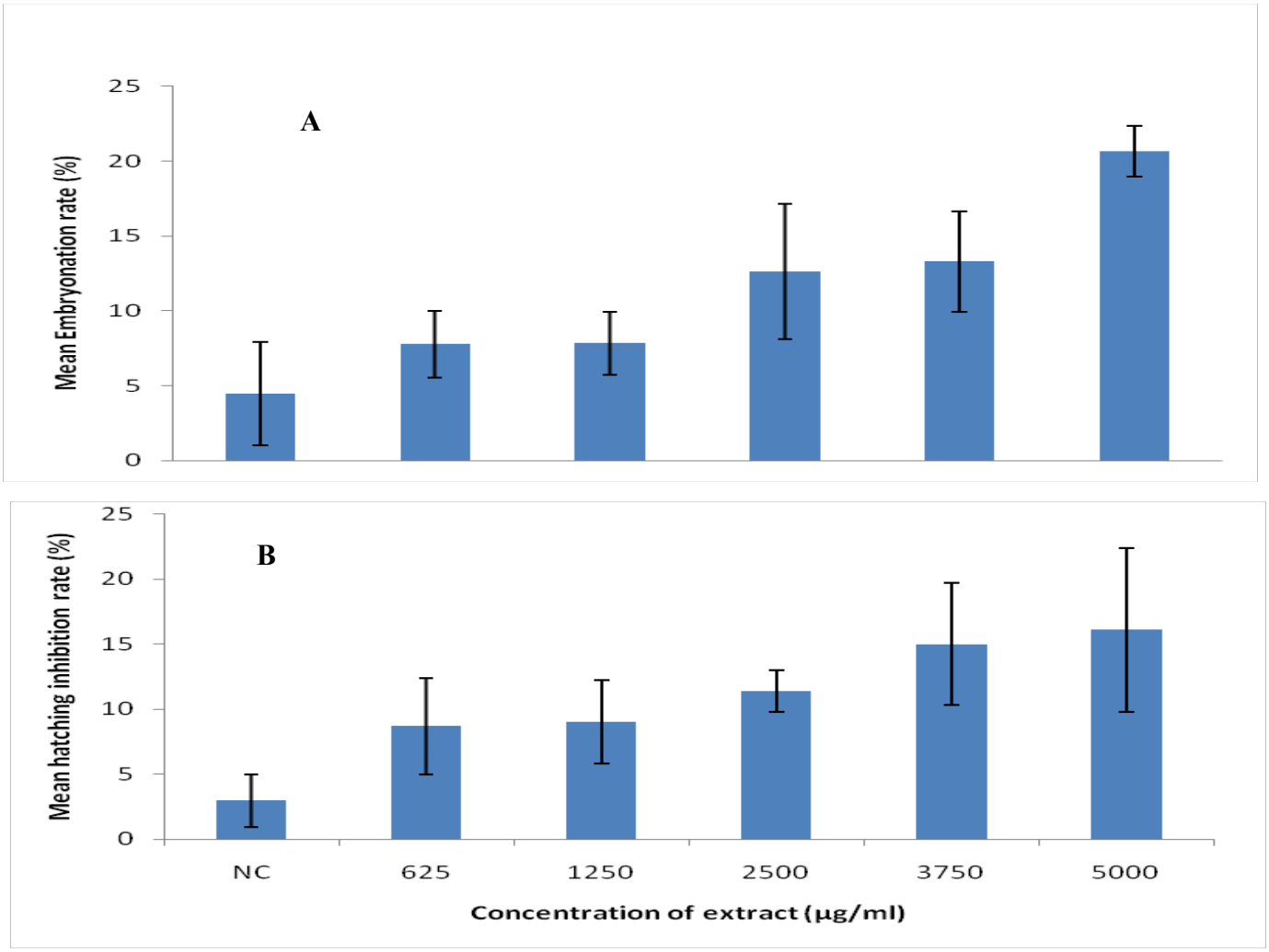

Figure 6 : Effect of *Annona senegalensis* extract on mean embryonation inhibition rate (A) and on mean hatching inhibition rate (B) of *Heligmosomoides bakeri* eggs. Legend: NC= Negative control

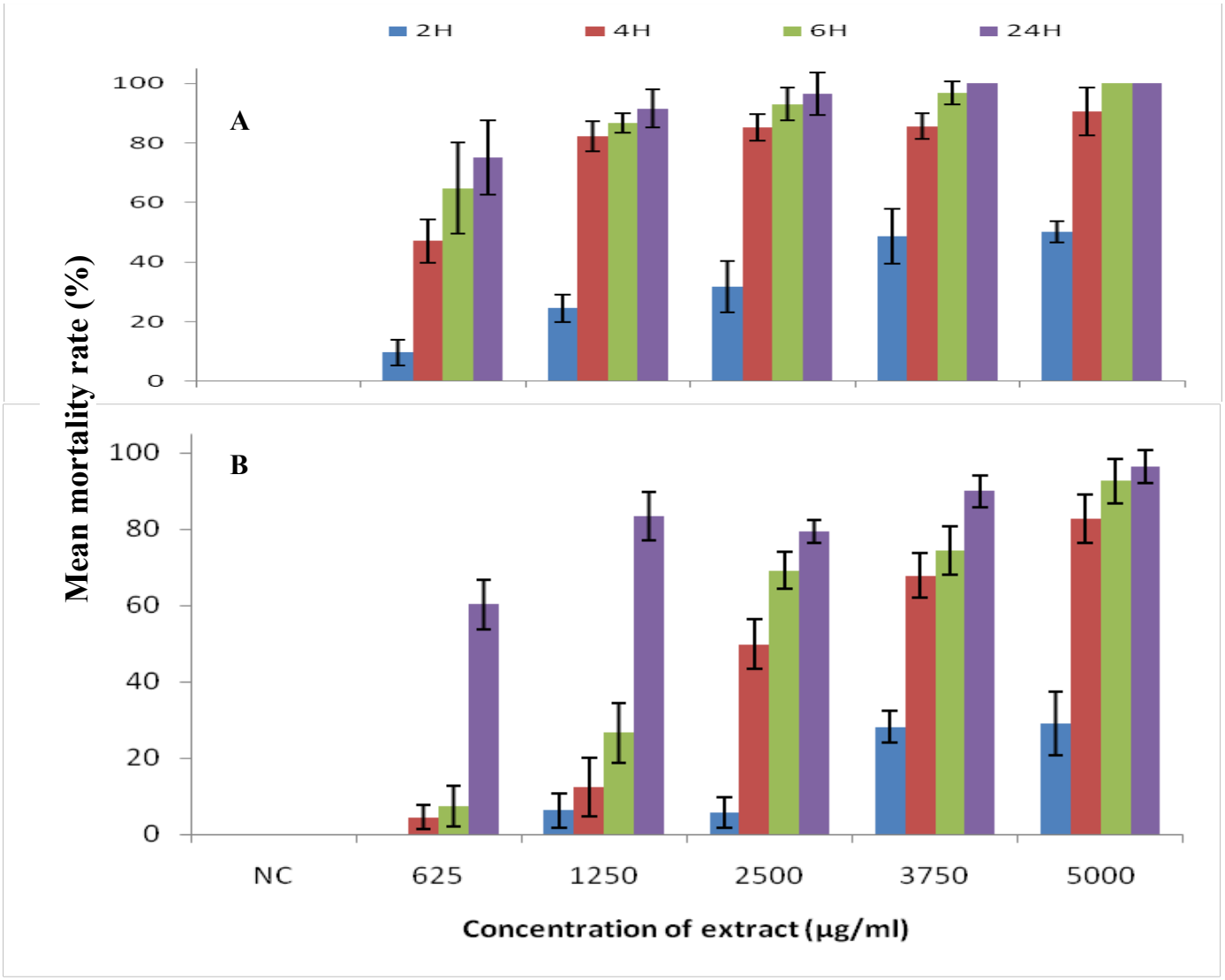

Figure 7 : Effect of *Annona senegalensis* extract on mean mortality rate of L1 larvae (A) and L2 larvae (B) of Heligmosomoides bakeri. Legend: NC= Negative control

V. Aknowledgements

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References Références Referencias

1. Annonymous 2010. "Annona senegalensis". AgroForestryTree Database (World Agroforestry Centre). Retrieved August 3, 2010.
2. Aké Assi, L., Guinko, S., 1991. Plantes utilisées dans la médicine traditionnelle en Afrique de l'Ouest. Editions Roche, Switzerland, p. 152.
3. Al-Qarawi, A.A., Mahmoud, O.M., Sobaih, M.A., Haroun, E.M., Adam, S.E., 2001. A preliminary study on the anthelminthic activity of Calotropis procera lates against Haemonchus contortus infaction in Najd sheep. Veterinary Research Communications 25, 61-70.
4. Argentieri PM., D'Addabbo T., Tava A., Agostinelli A., Jurzysta M., Avato P., 2008. Evaluation of nematicidal properties of saponins from Medicago sativa spp. Eur J Plant Pathol; 120, 189-197.
5. Bargere, I.A., 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. International Journal for Parasitology 29, 41-47.
6. Diehl, M.S., Kamanzi Atindehou, K., Téré, H., Betschart, B., 2004. Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria. Journal of Ethnopharmacology 95, 277-284.
7. Eguale T., Giday M. 2011. In vitro anthelminthic activity of crude extracts of five medicinal plants against egg hatching and larval development of

Haemonchus contortus. J Ethnopharmacol; 137, 108-113.
8. Fennel, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assesing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205-217.
9. Githiori, J.B., Hoglund, J., Waller, P.J., Leyden Baker, R., 2003. The anthelminthic efficacy of the plant, Albizia anthelmintica, against the nematode parasites Haemonchus contortus of sheep and Heligmosomoides polygyrus of mice. Veterinary Parasitology 116, 23-34.
10. Gqaleni, N., Moodley, I., Kruger, H., Ntuli, A., McLeod, H., 2007. Traditional and complementary medicine. In: Harrison, S., Bhana, R., Ntuli, A. (Eds.), South African Health Review. Health Systems Trust, Pretoria, South Africa.
11. Gray, G.D., 1997. The use of genetically resistant sheep to control nematode parasitism. Veterinary Parasitology 72, 345-357.
12. Hammond, J.A., Fielding, D., Bishop, S.C., 1997. Prospect for plant anthelminthics in tropical veterinary medicine. Veterinary Research Communications 21, 213-228.
13. Heng L., Koningsveld van JP., Roozen JP., 2004. Protein-flavour interactions in relation to development of novel protein foods. Trends Food Sci Technol; 15, 217-224.
14. Holmes, P.H., 1985. Pathogenesis of trichostrongylosis. Veterinary Parasitology 18, 89-101.
15. Kaboré A., Belem A.M.G., Tamboura H.H., Traore A., Sawadogo L., 2005. In vitro anthelminthic effects of two medicinal plants (Anogeissus leiocarpus and Daniellia oliveri) on Haemonchus contortus, on abomasal nematode of sheep in Burkina Faso. Afr. Journal Biotech. 8 (18), 4690-4695.
16. Kayser, O., Kiderlen, A.F., Croft, S.L., 2003. Natural products as antiparasitic drugs. Parasitological Research 90, S55-S62.
17. Lans, C., Brown, G., 1998. Observations on ethnoveterinay medicines in Trinidad and Tobago. Preventive Veterinary Medicine 35, 125-142.
18. Larsen, M., 1999. Biological control of helmiths. International Journal for Parasitology 29, 139-146.
19. Marie-Magdeleine C., Mathieu M., D'Alexis L., Philibert L., Archimede H., 2010a. In vitro effects of Tabernaemontana citrifolia extracts on Haemonchus contortus. Res Vet Sci; 89, 88-92.
20. Marie Magdeleine C., Udino L., Philibert L., Bocay B., Archimede H., 2010b. In vitro effect of cassava (Manihot esculenta) leaf extracts on four development stages of Haemonchus contortus. Vet Parasitol; 173, 85-92.
21. Middleton E., Kandaswami C., Theoharides T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol Rev; 52, 673-651.
22. Nchu F., Githiori JB., McGaw LJ., Eloff JN., 2011. Anthelminthic and cytotoxic activities of extract of Markhamia obtusifolia Spague (Bignoniaceae). Vet Parasitol; 183, 184-188.
23. Newton, S.E., Mun, E.A., 1999. The development of vaccines against gastrointestinal nematode parasite, particularly Haemonchus contortus [Review]. Parasitology Today 15, 116-122.
24. Nfi, A.N., Mbanya, J.N., Ndi, C., Kameni, A., Vabi, M., Pingpoh, D., Yonkeu, S., Mouss, C., 2001. Ethnoveterinary medicine in the northern province of Cameroon. Veterinary Research Communications 25, 71-76.
25. Ngangout Alidou M., Wabo Poné J., Khna Payne V., Komtangi Marie Claire, Yondo Jeannette, Mbogning Tayo Gertrude, Mpoame Mbida, Bilong Bilong C.F., 2012. Ovicidal and larvicidal activities of aqueous and ethanolic extract of stem bark of *Annona senegalensis* (Annonaceae) on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). Asian Pacific Journal of Tropical Biomedicine, 1-5.
26. Niezen, L.H., Robertson, H.A. Waghorn, G.C., Charlestone.G., 1999. Production, fecal egg counts and worms burdens of ewe lambs which grazed six contrasting forages. Veterinary Parasitology 80, 15-27.
27. Onyeyili, P.A., Nwosu, C.O., Amin, J.D., Jibike, J.I., 2001. Anthelminthic activity of crude aqueous extract of Nauclea latifolia stem bark against ovine nematodes. Fitoterapia 72, 12-21.
28. Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. International journal for Parasitology 29, 115-124
29. Satrija, F., Nansen, P., Murtini, S., He, S., 1995. Anthelminthic activity of papaya latex against Heligmosomoides polygyrus infections in mice. J. Ethnopharmacol. 48 (3), 161-164.
30. Schillhorn van Veen, T.W., 1997. Sense or nonsense? Traditional methods of animal parasitic disease control. Veterinary Parasitology 71, 177-194.
31. Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Veterinary Research 33, 509-529.
32. Van Wyk, J.A., Stenson, M.Mo., Van der Merwe, J.S., Vorster, R.J., Vilijoen, P.G., 1999. Anthelmintic resistance in South Africa: Surveys indicate an extremely serious situation in sheep and goat farming. Onder-stepoort Journal of Veterinary Research 66, 273-284.
33. Vieira, L.S., Cvalcante, A.R., Pereira, M.F., Dantas, L.B., Ximenes, L.F., 1999. Evaluation of anthelminthic efficacy of plants available in Ceara State, North-East Brazil, for the control of goat

gastrointestinal nematodes. Revue de Medicine Veterinaire 150, 447-452.
34. Wabo Poné J., Bilong Bilong C.F., Mpoame M., 2010. In vitro nematicidal activity of extracts of Canthium mannii (Rubiaceae), on different life-cycle stages of Heligmosomoides polygyrus (Nematoda, Heligmosomatidae). Journal of Helminthol. 84, 156-165.
35. Wabo Poné J., Bilong Bilong C.F., Mpoame M., Fusi Ngwa C., Coles C.G., 2006. In vitro activity of ethanol, cold and hot water extracts of the stem bark of Canthium mannii (Rubiaceae) on Ancylostoma caninum eggs. East & Central Afr. Journal Pharm. Sci. 9 (1), 14-18
36. Wabo Poné J., Olivia FT., Jeannette Y., Komtangi MC., Mbida M., Bilong Bilong CF., 2011. The in vitro effects of aqueous and ethanolic extracts of the leaves of Ageratum conyzoides (Asteraceae) on three life cycle stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). Vet Med Inter; 104061/ 2011 140293.
37. Wallace, D.S., Bairden, K., Duncan, J.L., Eckersall, P.D., Fishwick, G., Gill, M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M.J., 1998. The influence of dietary supplementation with urea on resilience and resistance to infection with Haemonchus contortus. Parasitology 116, 67-72.
38. Witty, M., 1999. Current strategies in the search for novel antiparasitic agents. International Jurney for Parasitology 29, 95-103.
39. Zajac, A.M., Gipson, T.A., 2000. Multiple anthelmintic resistance in a goat herd. Veterinary Parasitology 87, 163-172