academicJournals

Vol. 7(34), pp. 3220-3225, 17 November, 2013
DOI: 10.5897/JMPR2013.5160
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Nematicidal efficacy of methanol/methylene chloride extract of *Rauwolfia vomitoria* (Apocynacae) on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae) parasite of the white mouse (*Mus musculus*)

Jeannette Yondo[1]*, Marie-Claire Komtangi[1], Josué Poné Wabo[1], C. F. Bilong Bilong[3], Jules-Roger Kuiate[2] and Mbida Mpoame[1]

[1]Laboratory of Biology and Applied Ecology, Department of Animal Biology, Faculty of Science, University of Dschang, P O Box 067 Dschang, Cameroon.
[2]Laboratory of Microbiology and Antimicrobial Substances, Department of Biochemistry, Faculty of Science, University of Dschang, P O Box 067 Dschang, Cameroon.
[3]Laboratory of General Biology, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé 1, PO Box 812, Yaoundé, Cameroon.

Accepted 29 October, 2013

Parasitic diseases are the most common in tropical Africa where climatic and hygienic conditions favor their proliferation. Due to the limitations of synthetic therapy, there is a need to turn back to phytotherapy. Thus, the nematicidal efficacy of methanol/methylene chloride (v/v) extract of stem bark of *Rauwolfia vomitoria* (Apocynaceae) was tested on *Heligmosomoides bakeri*, a nematode parasite of *Mus musculus*. Seventy Swiss white mice of both sexes aged 5 to 6 weeks and weighing between 20 to 25 g were orally infected with a suspension containing about 100 to 110 one week old *H. bakeri* infective larvae (L_3). After the pre-patent period, infected animals were randomly divided into 7 groups of 10 animals each corresponding to the treatments. Thus, five doses of plant extracts [200, 400, 800, 1600, 3200 mg/kg body weight (bwt)] and a unique dose of 22 mg/kg bwt of albendazole were prepared. The nematicidal activity of the organic plant extracts compared to albendazole was assessed indirectly through faecal egg count reduction (FECR) and directly through total worm count reduction (TWCR). Albendazole served as the positive control while 4% dimethylsulfoxide (DMSO) as placebo. Each animal received for 6 consecutive days a daily dose of 0.7 ml of the organic plant extract according to its weight and group. Albendazole was given as a single dose. *R. vomitoria* at doses ≥ 1600 mg/kg bwt was active on the adult *H. bakeri* and reduced significantly ($P < 0.05$) the FEC and TWC of the nematode. The dose of 3200 mg/kg BW showed a high nematicidal activity of 76. 21% FECR and 79.55% TWCR, 7 days post-treatment. These results were similar to those obtained with albendazole, 88.67% and 85.60% respectively for FECR and TWCR. These findings suggest that *R. vomitoria* is an efficient anthelminthic. However, further studies are required in order to establish its mechanism of action. In addition, toxicological studies should be carried out.

Key words: Methanol/methylene chloride extract, *Rauvolfia vomitoria*, Albendazole, *Heligmosomoides bakeri, Mus musculus.*

INTRODUCTION

Helminthiasis is among the most important disease in both humans and livestock, causing considerable

hardship and stunted growth. In 2002, the world health organization (WHO) estimated that parasitosis represented more than 40% of the total morbid charge of diseases in the world (WHO, 2002). According to a 2004 report from the Cameroonian Public Health Secretary of State, of 16.1 million inhabitants who live in Cameroon, more than 10 million are infected by helminthiasis (Essogo, 2004). Parasitic diseases kill more than 155,000 people annually with 97% deaths occurring in developing countries (WHO, 2002; Hoste et al., 2006) and about 2 billions of people are infected (WHO, 2004). In the livestock sector, helminthiasis is among the most important animal diseases inflicting many economic losses such as low productivity and even mortality (Perry et al., 2002; Suleiman et al., 2005). To fight against helminthiasis, both humans and livestock farmers use synthetic drugs, which unfortunately present limitations such as high cost, irregular availability and side effects (Wabo et al., 2011b). The increasing resistance of parasites to chemotherapy (Cabaret et al., 2008) and the presence of residues in meat and milk (Gasbarre et al., 2001) have also been in concern in recent times. In this light, the search of alternative from nature can be justified (Paolini et al., 2003). Indigenous and herdsmen had long recognized the importance of helminth parasites and have made several attempts in controlling them through herbs (Suleiman et al., 2005). Several studies have been carried out to scientifically validate medicinal plants used by traditionalist to treat helminths (Mpoame and Essomba, 2000; Wabo et al., 2005, 2009, 2011a, b). According to tradipractitioners, *Rauvolfia vomitoria* (Apocynaceae) has several therapeutic properties (ICRA, 2008). It is distributed all over the world especially in Asia and in West and Central African countries. In Cameroon, especially in the “Bulu” and “Ewondo” speaking region, *R. vomitoria* is locally called “Medzanga Medzanga”. The plant has been used in lowering blood pressure (Amole et al., 2003) and as antimalarial remedy (Amole et al., 1993). It has also been reported to possess analgesic (Amole et al., 2006), haematinic (Amole and Ogunjere, 2001), antipyretic (Amole and Onabajo, 1999) and anticonvulsant properties (Amole et al., 2009). However, no information has been reported on the anthelmintic properties of *R. vomitoria*. Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols and reducing sugars. The alkaloids have been reported to include reserpine, a well-known antihypertensive substance found in this plant (Gubar, 1993). The aim of this work was to validate the anthelmintic activity of *R. vomitoria* (Apocynaceae) *in vivo*, using *Heligmosoides bakeri*, since the *in vitro* tests have already been carried out and organic extract of *R. vomitoria* showed a high mortality rate on larvae than aqueous extract. The *in vivo* test was carried out to determine the fecal egg count (FEC) and total worm count (TWC) reductions on the adult of *H. bakeri*. *H. bakeri* is a standard experimental model used in testing anthelmintic molecules by pharmaceutical companies (Githiori et al., 2003a). It belongs to the superfamily Trichostrongiloidea as most of the nematodes of veterinary importance (Githiori et al., 2003a) and its biological cycle is easily maintained in the laboratory on white mouse (*Mus musculus*).

MATERIALS AND METHODS

Plant

The stem bark of *R. vomitoria* (Apocynaceae) used in this work was obtained from traders in Yaounde, Center Region and political capital of Cameroon. *R. vomitoria* is a small tree or shrub (5 to 15 m in height and 40 cm in diameter) found in tropical or subtropical regions in Africa. The leaves are oval with straight venation and clusters of tiny flowers (Akpanabiatu et al., 2006a). The plant was identified at the National Herbarium of Cameroon (NHC) with the voucher numbers 16887.

Preparation of plant extracts

Five hundred grams (500 g) of plant powder was mixed with 3 liters of methanol/methylene chloride (1/1; v/v) for 3 days in a 5 liter jar. The mixture was stirred at least twice a day using a spatula, after which the mixture was filtered using a sieve of 150 µ of diameter, cotton and whatman no. 2 filter paper. The filtrate was concentrated using a rotary evaporator heated at 82°C for about 12 h. The wet extract collected was distributed in 5 large aluminum plates (22 cm of diameter). These plates were carefully placed in an oven and heated at 45°C for 2 days. The dried extract obtained was stored in air tight containers for further use (Ciulei, 1982) in the Laboratory of Applied Biology and Ecology (LABEA) of the University of Dschang. Two (2) g of extract was weighed and 0.8 ml of dimethyl sulfoxide (DMSO) added for better dilution of extract in water. The solution was completed to 20 ml with distilled water to obtain a final solution of concentration of 100 mg/ml. The quantity of the product received by each animal was determined using the following formula Vx = 0.032 bwt (where Vx is the volume of solution given to the animal, bwt, the body weight of the animal). This was administered to animals receiving the highest dose 3200 mg/kg bwt. For groups that received lower doses of 1600, 800, 400 and 200 mg kg^{-1} bwt, the quantity of the highest dose was divided by 2 in each subsequent group. The treatment was repeated for 6 days.

Preparation of the albendazole solution

From a tablet of albendazole brought in a local pharmacy, fifty (50) milligrams were diluted with 0.8 ml of DMSO and distilled water was added to obtain the final volume of 50 ml, thus a solution of concentration of 1 mg/ml. A single dose of 22 mg kg^{-1} bwt was administered using the following formula Vx = 0.022 bwt.

Artificial infection of mice with filariform larvae L_3 of *Heligmosomoides bakeri*

Seventy Swiss white mice aged 5 to 6 weeks and weighing 20 to 25

*Corresponding author: E-mail: yondojanet@yahoo.fr. Tel: (237) 77866549/97065619.

g were used for anthelmintic evaluation. They were randomly allocated into cages and allowed to acclimatize for one week. They had access to food and water *ad libitum*. Mice were inoculated orally with 100 to 110, one week old *H. bakeri* infective larvae (L_3) in 0. 8 ml volume of water. After mixing, 10 ml of solution containing larvae was withdrawn from the stock solution of which 0.1 ml was placed in a cover of Petri dish. The Petri dish was placed on top of the solution to immobilize the larvae which were then counted. Water was either added or removed until solution containing 13 to 15 L_3 larvae was obtained. The mice were infected orally by gavage, using a blunt ended needle (Enriquez et al., 1993). The parasite strain used in this work was provided by Pr J. B. Githiori of the National Veterinary Institute, Nairobi, Kenya. These larvae were afterwards maintained through passage in a colony of inbred CBA strain mice at the Laboratory of Applied Biology and Ecology (LABEA) of the University of Dschang, Cameroon, according to procedures outlined by Githiori (2004).

Experimental design

After the pre-patent period (9 to 11 days), mice were isolated in individual cages to collect fecal pellets. The feces were homogenized in a mortar and a saturated salt solution added. After filtration through a sieve, the supernatant was used to fill 2 test tubes until the superior meniscus was formed. A cover slide was gently lowered and allowed for 5 min, after which cover slide was removed and placed on a slide. Eggs were counted at 4× objective using a light microscope. As soon as the qualitative coproscopic analyses showed evidence of infection (presence of eggs in faeces of mice), animals were randomly allocated into 7 groups of 10 individuals each (a pilot study was carried out before establishing doses 200, 400, 800, 1600 and 3200 mg kg^{-1} bwt). Each mouse was weighed prior to treatment and dosed based on the individual's body weight early in the morning each day for 6 consecutive days. The volume of tested product was slowly administrated orally using a manual syringe of 1 ml capacity with a blunt end needle. Groups 1 to 5 were treated orally with the stem bark extract of plants at different doses for 6 consecutive days, while group 6 was treated with a single dose of albendazole (22 mg kg^{-1} bwt) as standard anthelmintic (positive control). Group 0 received 4% DMSO which served as negative control. FEC was taken for each mice first thing in the morning before treatment on days 0, 3, 5 and 7 post treatments for each group. At the end of the treatment (day 7), the parasite burden (total worm count or TWC) was determined.

Evaluation of faecal egg count reduction (FECR)

After the prepatent period of 9 to 11 days, before and during the treatment (on days 0, 3, 5 and 7), mice were individually placed in labeled cages without bedding or food for 30 min early in the morning. The fecal pellets produced were immediately collected with a teaspoon and placed in labeled Petri dishes containing some distilled water to prevent fecal materials from drying out. The standard procedure consists of mixing 2 g of feces with 60 ml of saturated salt solution (Thienpont et al., 1979). When the quantity of faeces was less than 2 g, the amount of saturated salt solution was proportionately reduced. The faeces were homogenized in a porcelain mortar and suspended in the mentioned salt solution. Aliquots were examined under a microscope (4× magnification) using Mac Master egg counting slide with a sensitivity of 50 eggs per gram (EPG). The faecal egg count reduction (FECR) was determined by the following formula (Coles et al., 1992):

FECR (%) = 100 (1- T/C)

Where T and C are the geometric means of FEC in the treated and control groups, respectively.

Evaluation of total worm count reduction (TWCR)

Seven days post-treatment mice were euthanized using chloroform. The body cavity was opened to remove the small intestine which was placed in a large Petri dishes containing 20 to 30 ml of distilled water. This organ was opened longitudinally with a scissors and passed through the arms of a forceps. The exudates containing parasites was washed in the water and all worms recovered were counted under a dissecting microscope. The percentage of total worm count reduction (TWCR) was calculated using the following formula (Enriquez, 1993):

$$\text{TWCR (\%)} = 100 \times \frac{\text{Total worm count in control - Total worm count in treated group}}{\text{Total worm count in control group}}$$

Statistical analysis

Statistical analysis of data was performed using two-way analysis of variance (ANOVA) followed by Waller-Duncan test for comparison between groups. Egg per gram of faeces (EPG) was analysed after its transformation into log (x + 1). The comparisons of means of fecal egg count (FEC) and total worm count (TWC) were done at $p < 0.05$. All analyses were performed using SPSS 19.0 program.

RESULTS AND DISCUSSION

Feacal egg count reduction (FECR)

Results on FECR are shown on Table 1. The negative control (4% DMSO) group showed increase in egg counts throughout the treatment period. This increase was highly significant ($p < 0.05$) when compared with groups that received the plant extract and albendazole. Albendazole showed significant reduction ($p < 0.05$) from day 3 post treatment, but this reduction was not significant after day three. A similar trend was observed with plant extract, where reduction observed on the third day after treatment was not significantly different ($p > 0.05$) when compared with days 5 and 7. The highest FECR rate was obtained with albendazole (88.67%), followed by 77.23% reduction with plant extract at 3200 mg/kg bwt. FECR was dose dependent.

Effects of methanol/methylene chloride extract and albendazole on the parasitic intensity of the nematode/Total worm count reduction (TWCR)

For the TWCR, albendazole was the most effective (85.6%), while *R. vomitoria* produced 79.6% reduction in TWC at 3200 mg/kg bwt (Table 2). These results were not significantly ($p > 0.05$) different when compared statistically. The results showed that the TWC was dose dependent. The reduction in FEC and TWC observed in this study could be explained by the fact that when adult parasites come in contact with the product, their

Table 1. Mean variation ± standard deviation and the percentage of fecal egg count reduction (at day 3, 5 and 7) after treatment with 4% dimethylsulfoxide (DMSO), albendazole and different doses of methanol/ methylene-chloride extract of *Rauvolfia vomitoria*.

Treatment	Period of treatment (days)				
	Dose (mg/kg bwt)	DO	D3	D5	D7
4% DMSO (negative control)	-	11000±8700	30570±8700[a] (0.0)	28550±2740[a] (0.0)	42820±4500 (0.0)[a]
Albendazole (positive control)	22	14850±2980	2500±1500[e] (89.69±10.33)	3920±110[e] (83.81±13.35)	2620±350[e] (88.67±10.48)
	200	15500±2040	24600±7480[b] (34.18±3.43)	28900±6550[b] (33.19±3.79)	34250±5170[b] (20.64±2.29)
Rauvolfia vomitoria	400	5400±2420	13500±5050[b] (39.85±5.46)	14800±5670[b] (37.68±4.14)	19800±4110[b] (22.85±4.51)
	800	8900±3320	11300±6000[c] (51.33±3.19)	18200±1470[c] (47.94±2.23)	19300±1400[c] (46.49±5.30)
	1600	25200±6130	13400±2680[d] (60.22±5.16)	13200±3820[d] (60.24±5.13)	13100±3230[d] (61.22±5.14)
	3200	14600±3040	7100±3470[e] (75.98±3.57)	6500±3550[e] (77.23±8.02)	6900±920[e] (76.21±4.67)

a, b, c, d and e numbers with the same letter in the same column are not significantly different (p>0.005). D: day; FECR: faecal egg count reduction; DMSO: Dimethilsulfoxide; ( ): Percentage of fecal egg count reduction.

Table 2. Mean variation± standard deviation of total worm count and the percentage of total worm count reduction (at day 7) after treatment with 4% dimethylsulfoxide, albendazole and different doses of methanol/ methylene-chloride extract of *R. vomitoria*.

Treatments	Doses (mg.kgbwt^{-1})	TWC	TWCR
4%DMSO (negative control)	-	96±10	-
Albendazole	22	15.3±7	85.6[e]
	200	64±15	32.8[a,b]
	400	53.8±14	43.6[a,b,c]
Rauvolfia vomitoria	800	49.8±9	59.4[c]
	1600	28.5±12	74.8[d]
	3200	23.5±13	79.6[d,e]

a, b, c, d and e numbers with the same letter in the same column are not significantly different at p < 0, 05. TWC: total worm count; TWCR: total worm count reduction; DMSO: dimethilsulfoxide; -: not determined.

metabolism is affected and some of the parasites die, thus leading to reduction in egg out-put.

Phytochemical screening of *R. vomitoria* revealed the presence of secondary metabolites such as polyphenolic compounds, tannins, alkaloids and flavonoids (Yondo et al., 2009). The stem barks of

plants are the site of storage of secondary metabolites responsible for the biological properties of medicinal plants extracts (Busindou, 1997). Polyphenolic compounds are known to inhibit parasite growth by causing apoptotic cell death, leading to the reduction of parasite and egg burdens (Inuoe, 1994; Rice, 1965; Adedapo et al., 2005). Tannins interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997), while alkaloids and flavonoids are good sources of chemicals responsible for the therapeutic activities of several medicinal plants (Debella, 2002). Flavonoids mode of action is linked to its unusual antioxidant pathway. Ribeiro et al. (1997) discussed that the lack of defense mechanisms against oxidative stress makes the parasites susceptible to drugs having an effect on the generation of reactive oxygen species. The increase in egg out-put after reduction may be due to the fact that parasites that did not die may show some level of resistance to the product, and start laying eggs.

Results of this study were consistent with that of Satrija et al. (1995) and Wabo et al. (2009) who tested the efficacy of *Carica papaya* and *Canthium manii*, respectively against this same parasite (*H. bakeri*).

Inhibition on egg production is an important aspect of the activity of some antinematodals as benzimidazoles and phenothiazines. Albendazole has a large broad spectrum on many parasites of medico-veterinary importance (Lagardère, 1995). Similar studies carried out by Onyeyili et al. (2001) shows a percentage of reduction of faecal count of 94.1% with this proprietary anthelmintic against mixed nematode species (*Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Oesophagostomum columbianum*, *Strongyloides papillosus* and *Trichuris ovis*) at the dose 5 mg kg bwt^{-1} on the sheep. This mouse host-parasite model used was suitable for a rapid evaluation of anthelminthic effect of *R. vomitoria* in a relatively short period. So, the reduction rate above 70% obtained puts this plant as a potential source of anthelmintic (Githiori et al., 2003b).

From the work that was aimed at assessing the anthelminthic activity of organic extract of *R. vomitoria*, we noticed that the methanol/methylene chloride extract of *R. vomitoria* has generated a high reduction rate of the ovary charge (76.21%) and parasitic load (79.55%) of the adults of *H. bakeri*. The effect of extract was dose dependent. Further research such as purification and identification of bioactive compounds and toxicological investigation are necessary.

ACKNOWLEDGMENTS

The authors wish to express their thanks to the traditional herbal traders of Mokolo market in Yaoundé, who provided us with the stem barks. We also thank the Laboratory of Nutrition and Nutritional Biochemistry, University of Yaoundé 1, Cameroon, particularly Mr. Fomekong Dongmo Gilles Ines, who helped us with plant extractions.

REFERENCES

Adedapo AA, Shabi OO, Adedokun OA (2005). Anthelmintic efficacy of the aqueous crude extract of Euphorbia hirta Linn in Nigerian dogs. Vet. Arhiv. 75:39-47.

Akpanabiatu MI, Umoh IB, Eyong EU (2006a). Influence of *Rauwolfia vomitoria* root bark extract on cardiac enzymes of normal Wistar albino rats. Biopharmaceut. J. 14:273-278.

Amole OO (2003). Blood pressure responses to aqueous extract of *Rauvolfia vomitoria* (Afzel). Niger. J. Health Biomed. Sci. 2:50-51.

Amole OO, Agbaje EO, Onabanjo AO (1993). Chemotherapeutic actions of *Rauvolfia vomitoria* on *Plasmodium yoelii* infection. Niger. J. Physiol. Sci. 9:35-38.

Amole OO, Ogunjere OO (2001). Evaluation of the haematological properties of the plant extract of *Rauvolfia vomitoria* (Afzel) J. Med. Sci. 3:14-15.

Amole OO, Onabanjo AO (1999). Antipyretic effect of the extract of *Rauvolfia vomitoria* (Afzel) in rabbits Nig. J. Nat. Prod. Med. 3:77-78.

Amole OO, Onabanjo AO, Odofin AA (2006). The analgesic effect of *Rauvolfia vomitoria* (Afzel). Biomed. Res. 17:125-127.

Amole OO, Yemitan OK, Oshikoya KA (2009). Anticonvulsant activity of *Rauvolfia Vomitoria*. Afr. J. Pharm. Pharmacol. 3:319-322.

Busindou M (1997). Investigation on traditional phytotherapy at Kindamba and Odzala (Congo) and analysis of convergence of the use of medicinal plants in Central Africa. Doctoral thesis, university of Bruxelles p. 482.

Cabaret J, Charvet C, Fauvin A, Silvestre A, Sauve C, Cortet J, Neveu C (2008). Gastrointestinal strongyles of ruminants: Mechanism of anthelminthic resistance and consequences on their management. C. France — 2009 - Tome 162 - N°1 http://www.academie-veterinaire-defrance.org. Bull. Acad. Vét. 162:33-38.

Ciulei I (1982). Methodology for analysis of vegetable drugs. Practical manual on the industrial utilization of medical and aromatic plants. Bucharest, Romania p. 67.

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor M, Waller PJ (1992). World Association for the advancement of the Veterinary Parasitology (W. A. A. V. P) methods for the detection of the anthelmintic resistance in nematodes of veterinary importance. Vet. Par. 44:35- 44.

Debella A (2002). Manual for phytochemical screening of medicinal plants. Ethiopian health and nutrition Research Institute. Addis Ababa, Ethiopia.

Enriquez JB (1993). Les médicaments anthelminthiques utilisés en médecine des carnivores domestiques. Activité et toxicité. Rec. Méd. Vét. 189:499-512.

Essogo A (2004). Croisade contre les vers intestinaux. www.allafrica.com. p. 1.

Gasbarre LC, Stout WL, Leighton EA (2001). Gastrointestinal nematodes ofcattle in the-eastern US: Results of a producer survey. Vet. Parasitol. 101:29-44.

Githiori JB (2004). Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and smallholger farmers in Kenya. Doctoral thesis. Department of Biomedical Sciences and Veterinary Public Health, SLU. Acta universitatis Agricultural Sulciae p. 76.

Githiori JB, Höglund J, Waller PJ, Baker RL (2003a). The anthelminthic efficacity of the plant, *Albizia anthelminthica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoïdes polygyrus* of mice. Vet. Parasitol. 116:23-34.

Githiori JB, Höglund J, Waller PJ, Baker RL (2003b). Evaluation of anthelminthic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmer in Kenya against *Heligmosomoïdes polygyrus* infections in mice. Vet. Parasitol. 118:215-226.

Gubar SI, Konstantinova EP, Kunakh VA (1993). Medicinal and aromatic plants for Human Welfare WOCMAP: Botanical Aspects, Genetic Resources, Genetics, Breeding, Biotechnology July 19-25, 1992, Maastricht, Netherlands. Acta Horticult. pp. 281-286.

Hoste H, Jackson F, Athanasiadou S, Thamsborg MS, Hoskin OS (2006). The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 22:253-261.

International Center for Research in Agroforestry (ICRA) (2008). AgroForestryTree database: a tree species reference and selection guide (online resource). www.worldagroforestrycentre.org.

Inuoe M, Suzuki R, Koide T, Sakaguchi N, Ogihara Y, Yabu Y (1994). Antioxidant, gallic acid, induces apoptopsis HL-60 RG cells. Biochem. Biophy. Res. Commun. 204:898-904.

Lagardère B (1995). Pédiâtre hôpital Ambroise-Paris, Boulogne sur Seine. Développement et Santé numéro 117, juin 1995.

Martin RJ (1997). Mode of action of anthelmintics drugs. Vet. J. 154:11-34.

Mpoame M, Essomba LI (2000). Attempt of treatment against gastrointestinal parasitosis of domesticated fowl using aqueous decoctions of seeds of papaya (*Carica papaya*). Rev. Elev Méd. Vét. Pays Trop. 53:23-25.

Onyeyili PA, Nwosa CO, Amin JD, Jibike JI (2001). Anthelmintic activity of crude aqueous extract of Nauclea latifolia stem baark against ovine nematodes. Fitoterapia 72:12-21.

Paolini V, Frayssines A, De La Farge F, Philippe D, Hervé H (2003). Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. Vet. Res. 34:331-339.

Perry B, Randolf TF, McDermont JJ, Sones KR, Thornton (2002). Investing in Animal Health Research to alleviate Poverty. International Livestock Research Institute (ILLRI): Nairobi, Kenya p. 148.

Ribeiro A, Piló-Veloso D (1997). Trypanosomal flavonoids from *Trixis vauthierie*. J. Nat Prod. 60:836-841.

Rice EL (1965). Inhibition of nitrogen-fixing and nitrifying bacteria by seed plant II. Characterization and identification of inhibitors. Plants Physiol. 18:255-268.

Satrija F, Nansen P, Murtini S, He S (1995). Anthelminthic activity of papaya latex patent *Heligmosomoïdes polygyrus* infections in mice. J. Ethnopharmacol. 48:161-164.

Suleiman M, Mamman M, Aliu YO, Ajanusi JO (2005). Anthelmintic activity of the crude methanol extract of Xylopia aethiopica against *Nippostrongylus brasiliensis* in rats. Vet. Arhiv. 75:487-495.

Thienpont D, Rochette F, Vanparijs O (1979). Diagnostic des verminoses par examen coproscopique. Beerse, Belgique, Janssen Res. Foundation p. 187.

Wabo PJ, Bilong BCF, Mpoame M (2009). *In vitro* nematicidal activity of extracts of *Canthium manii* (Rubiaceae), on different life-cycle stages of heligmosomoides polygyrus (Nematoda, heligmosomatidae). J. Helminthol. 84:156-165.

Wabo Poné J, Fossi Tankoua O, Yondo J, Komtangi MC, Bilong BCF, Mpoame M (2011b). 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 *Heligmosoides bakeri* (Nematoda, HeligmosomatidaeArticle ID 140293, doi:10.4061/2011/140293. Vet. Med. Int. p. 5.

Wabo PJ, Mpoame M, Bilong BCF, Kerboeuf D (2005). The *in vitro* comparative study of anthelminthic activity of ethanolic extract of stem bark of *Canthium manii* (Rubiaceae) and Mebendazole. Rev Méd Vét. 15:633-636.

Wabo PJ, Yondo J, Fossi Tankoua O, Komtangi MC, Bilong BCF, Mpoame M (2011a). The i*n vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). J. Pharm. Phyto. 3:1-5.

WHO (World Health organisation) (2002). Promotion de la médecine traditionnelle dans le système de santé. Stratégie de la région africaine, Harare, Zimbabwe p. 20.

WHO (world Health organisation) (2004). La schistosomiase et les géohelminthiases: action de prévention et de lutte. www.who.intl.

Yondo J, Fomekong DGI, Komtangi MC, Wabo PJ, Fossi TO, Kuiate JR, Mpoame M (2009). *In vitro* antioxidant potential and chemical constituents of three Cameroonian medicinal plants used to manage parasitic diseases. Pharmacologyonlin 1:648-657.