

PRELIMINARY STUDIES ON THE ANTHELMINTIC EFFECTS OF ETHANOLIC EXTRACT OF *GARCINIA KOLA* (HECKEL) SEED AND METHANOLIC EXTRACT OF *SACOGLOTTIS GABONENSIS* (BAILLON) STEM BARK ON *HELIGMOSOMOIDES BAKERI* LARVAE IN NSUKKA, NIGERIA

I. W. ONYENWE, L. A. NGONGEH, C. C. UDEKWU AND G. O. EZEUGWU

(Received 9, June 2009; Revision Accepted 20, November 2009)

ABSTRACT

The anthelmintic activities of two folkloric medicinal plants, *Garcinia kola* (Heckel) seed and *Sacoglottis gabonensis* (Baillon) stem bark were investigated independently in *in vitro* studies. The effects of the crude ethanolic and methanolic extracts of the two plants, on the pre-parasitic stages of *Heligmosomoides bakeri*, in egg-hatch assay for *Garcinia kola* and larval mortality assay for both plants, were studied. Three down-graded concentrations, (100, 50 and 25 mg/ml) of *Garcinia kola* were used in both assays. The larval mortality assay for *Sacoglottis gabonensis* was carried out with the plant extract concentrations of, 125, 62.5, 31.5 and 15.625 mg/ml, and the larval counts were determined at specified time interval of 30 minutes. The concentrations, 25, 12.5 and 6.25 mg/ml of two standard anthelmintics, Albendazole and levamisole, were used in the positive control test of the egg-hatch and the larval mortality assays respectively, while of the *Garcinia kola* seed, 25 mg/ml of levamisole was used in the positive control test of the *Sacoglottis gabonensis* study. Distilled water was used in the negative control for both assays. Every test was set up in triplicate. By using the number of eggs that hatched in distilled water as the standard, the number of eggs that hatched in each concentration of the plants extracts and the drugs was expressed as a percentage reduction in egg-hatch. The same procedure was used to obtain the percentage paralysis of the larvae in the larval mortality assay. *Garcinia kola* seed extract inhibited only 18.75% of the egg-hatch at the maximum concentration used but irreversibly paralyzed 76.52% of the larvae at 50 mg/ml. This compares with the efficacy of levamisole at 12.5 mg/ml; ($p > 0.05$). The maximum and minimum concentrations of *Sacoglottis gabonensis* achieved 100 % irreversible larval paralysis, compatible with the 25 mg/ml of levamisole, in 30 and 150 minutes respectively. Both plant extracts were found to possess some anthelmintic activities *in vitro* that could further be exploited.

KEY WORDS: *Garcinia kola*, *Sacoglottis gabonensis*, Anthelmintic, *in vitro*

INTRODUCTION

Helminthiasis is one of the major disorders that adversely affect animal production. Several anthelmintics had been produced for the treatment of helminthiasis, but a major set back for their continued usage is the development of resistance (Asuzu and Njoku, 1996). Overdependence on drugs, frequent treatments and misuse of chemical-based anthelmintics has led to the development of anthelmintic resistance, particularly in sheep and goats (Jackson, 1993; Prichard, 1994). Furthermore, some of the drugs have been found to have intolerable levels of toxicity in the ecosystem (Chema and Ward, 1990). Therefore, according to Chiejina *et al.* (2002), there is a great need for a more sustainable alternative control strategy which must not only be affordable and available to the small-scale farmers, in the traditional system of production, but also have a simple mode of administration.

Various folkloric plant products, available in Nigeria, had always been used in the traditional treatment and management of suspected cases of helminthiasis in animals in the rural areas. However, the

number of investigative studies done on the medicinal properties of these plants has so far remained low, despite the availability of enormous biodiversity resources in Nigeria. Among the folkloric anthelmintics being used by the rural people in the Southeast Nigeria are *Garcinia kola* & *Sacoglottis gabonensis*. The *Garcinia kola* (Heckel) is a tropical tree of the family Guttiferae (Keany *et al.*, 1964). It is a medium-sized tree that can grow to a height of about 12 m. It is commonly found in the forest regions of West and Central Africa. The edible seed is eaten raw and it is widely known as "Bitter kola". It is however, known by different names among the various linguistic tribes of West Africa. E.g., in Nigeria, it is known as *Orogbo* in the Yoruba-speaking Southwest; *Aku-ilu* or *Ugoro* among the Igbos of the Southeast and *Naminjigoro* by the Hausas of the Northern part (Nwosu *et al.*, 2004) In most parts of Liberia, it is called *Basawa-meh* while it is commonly called *Ngai* and *Mcsagbior sabel* in Cameroun and Sierra Leone, respectively (Keany *et al.*, 1964 and Nwosu *et al.*, 2004). Most parts of the plant are claimed, in traditional medicine, to be efficacious against diarrhoea, dysentery, bronchitis, hepatitis, asthma, sore-

I. W. Onyenwe, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria
L. A. Ngongeh, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria
C. C. Udekwu, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria
G. O. Ezeugwu, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria

throat, gastrointestinal helminthiasis, e.t.c. The active constituents in the seed are flavonoids, xanthenes, benzophenones and alkaloids (Ebong and Karuba-Owiyi, 1996; Nwosu *et al.*, 2004).

Sacoglottis gabonensis (Baillon) Urb. Humiriaceae is a large tropical rain forest tree found along the coastal states of Nigeria. The bark is reddish-brown in colour. The bark scrapings are principally used as palm-wine additive by the locals to improve the shelf-life of the wine. It also gives an amber colour and a different taste to the wine. The folkloric claims on the efficacy of this plant's bark on the intestinal and palm wine micro flora had been investigated by Faparusi and Osiyemi, (1973). Certain phytochromes that were identified in *Sacoglottis gabonensis* are tannins (Ethylgallates), 1, 2 - naphthoquinone and two uncharacterized phenol compounds (Maduka and Okoye, 2003). The only study that had been done on the anthelmintic claims of these two plants was by Nwosu *et al.*, (2001 and 2004), using their crude aqueous extracts against ruminants nematodes. This work is to investigate the anthelmintic effect of the ethanolic crude extract of *Garcinia kola* seed and the methanolic crude extract of *Sacoglottis gabonensis*.

MATERIALS AND METHODS

Collection and preparation of the plant materials

The stem bark of *Sacoglottis gabonensis* and *Garcinia kola* seeds were obtained from the local markets in Aba, Abia state and Nsukka, Enugu state, respectively, in Nigeria. They were identified by the Curator/Taxonomist of the Herbarium of the Bioresources Development and Conservation Programme (BDGP) centre, Nsukka, Nigeria. The plant materials were separately dried, under mild sunlight, to a constant weight for 7 days. Each was shredded into bits, crushed in a mortar and finally pulverized in a hammer-mill with an in-built coarse sieve to obtain the dry powder.

Extraction of the plant materials

100 gm each of *Garcinia kola* and *Sacoglottis gabonensis* powder were extracted in 1000 ml of 50% ethanol and methanol respectively, in stoppered glass jars. They were left on the bench at room temperature for 48 hours with a 6-hourly intermittent vigorous shaking. Double filtration was done with Whatman filter papers. The filtrates were evaporated to dryness at 40 °C in a hot-air oven. The dried extracts were stored at 4 °C in airtight bottles and used within one week.

Egg-hatch assay with *Garcinia kola* seed extract

Faecal egg culture pastes were set up in 21 petri dishes by smearing washed faecal pastes from infected mice on the filter papers, the filter paper retains moisture for the culture. Each paste contains 300-350 eggs of *Heligmosomoides polygyrus (bakeri)* from freshly voided faeces of experimentally infected albino mice. The down-graded concentrations, (100, 50 and 25mg/ml) of the *Garcinia kola* seed extract were used to moisten the faecal paste, in triplicate, for each concentration. Similarly, three down-graded concentrations, (25, 12.5 and 6.25 mg/ml) of the standard anthelmintic drug, Albendazole, also in triplicates, were used as the positive control. The last

three dishes were moistened with distilled water as the negative control. The dishes were covered and incubated in the refrigerator at 4 °C for 7 days.

Larval recovery and count

The larvae from the hatched eggs were recovered by gently flooding the faecal paste with distilled water to facilitate larvae emergence. The dishes were anchored at a 45° gradient to ease the downward flow of the emerging larvae for 5 minutes (Fakae *et al.*, 1994). The larvae were aspirated into clean calibrated petri dishes for counting, using a stereomicroscope with a light source. The value from each count was expressed as a percentage reduction in the egg-hatch by using the value from the distilled water (negative control) as the standard, which is expressed as 0% reduction.

Larval mortality assay

Freshly recovered larvae from normal faecal cultures of albino mice experimentally infected with *Heligmosomoides polygyrus (bakeri)* were used in this test. Pilot trials had earlier been done to estimate the duration of the activity of the different concentrations of the extracts, and the standard anthelmintic which was used as the positive control.

a) Test with *Garcinia kola* seed extract:

12 motile larvae were delivered in 0.4 ml of distilled water into each of 21 wells of a microtitre plate. Adjustments were made to obtain extract concentrations of 100, 50 and 25 mg/ml, in the wells in triplicates, respectively. A similar process was repeated with the standard anthelmintic drug, levamisole, using the concentrations of 25, 12.5 and 6.25 mg/ml as the positive controls. The last three dishes containing distilled water, of equal volume, were kept as the negative control. The plates were covered and kept in the refrigerator at 4 °C for 24 hours before the single count.

b) Test with *Sacoglottis gabonensis* stem bark:

20 motile larvae were delivered in 0.4ml of distilled water into 18 wells of a microtitre plate. Adjustments were similarly made to obtain 125, 62.5, 31.5 and 15.625 mg/ml concentrations of the extract, each in triplicate. Only 25 mg/ml of the standard drug, levamisole, in triplicate was used as the positive control. The remaining three wells with distilled water of equal volume were kept as the negative control.

c) The larval count:

A stereomicroscope with a light source was used for the count. A larva is classified as motile (if there is an observable movement) or paralysed/dead (if there is none) during a 5-second interval of exposure to the source of light, according to the technique of Martin and Le Jambre (1979). The counts for the *Sacoglottis gabonensis* test were done at a 30 - minute interval for duration of 150 minutes. The process was repeated after 24 hours in each well, for the two tests, to check for any case of reversal of paralysis.

The results were interpreted using the Analysis of Variance to evaluate the level of significance between the parameters measured.

RESULTS

The extract yields were 24% and 21% (w/w) for *Garcinia kola* seed and *Sacoglottis gabonensis* stem

bark, respectively. At the highest concentration of the *Garcinia kola* seed (100 mg/ml) used, only 18.75% of the egg hatch was inhibited whereas the standard drug,

Albendazole's lowest concentration of 6.25 mg/ml inhibited 98.88% of the eggs. Both exhibited a dose-dependant activity as shown in Table 1.

Table 1: Egg-hatch assay with *Garcinia kola* seed extract showing number of hatched eggs and the percentage reduction in egg-hatch

Treatment	Mean no. of eggs hatched	Range	% reduction in egg-hatch
Extract (mg/ml)			
100	216.67 ± 5.77 ^a	208 -222	18.75
50	240.00 ± 10.00 ^b	230 -250	10.00
25	260 .00 ± 20.00 ^c	238 -384	2.50
Albendazole (mg/ml)			
25	0.00 ^d	0	100
12.5	1.00 ± 1.00 ^d	0 - 2	99.63
6.25	8.33 ± 0.57 ^e	8 - 9	96.88
Distilled water	266.67 ± 20.82 ^c	250 - 290	0.00

*The value for the distilled water was used as the standard, and expressed as 0.00% reduction in egg-hatch
Values with different superscripts are significantly different (P < 0.05)

Table 2: Larva mortality assay with *Garcinia kola* seed extract showing the number and percentage of paralysed larvae

Treatment	Mean no. of paralysed larvae	Range	Percentage paralysed
Extract (mg/ml)			
100	9.00 ± 1.00 ^a	8 - 9	79.44
50	8.67 ± 0.58 ^a	8 - 9	76.52
25	5.33 ± 1.53 ^b	4 - 7	47.04
Levamisole (mg/ml)			
25	11.33 ± 0.58 ^c	10 -12	100
12.5	8.67 ± 0.58 ^a	8 - 9	76.52
6.25	6.00 ± 1.00 ^d	5 - 7	52.96
Distilled water	0.67 ± 0.58 ^e	0 -1	*0.00

*The value for the distilled was used as the standard, and expressed as 0% paralysis of the larvae
Values with different superscripts are significantly different (P < 0.05)

Table 3: Larva mortality assay with *Sacoglottis gabonensis* stem bark extract showing the duration of activity, number and percentage of paralysed larvae.

Time (mins)	Extract concentration in mg/ml				Levamisole (25 mg/ml)	Distilled water
	125	62.5	31.25	15.625		
30	20 (100%)	17.7±1.53 ^a (88.5%)	12.0±2.0 ^b (60%)	7.3±1.53 ^c (36.5%)	20 (100%)	0 (0%)
60	20 (100%)	20 (100%)	15.3±1.53 ^d (76.5%)	10.3±2.10 ^e (51.5%)	20 (100%)	0 (0%)
90	20 (100%)	20 (100%)	19±1.0 ^f (95%)	14.7±0.58 ^g (73.5%)	20 (100%)	0 (0%)
120	20 (100%)	20 (100%)	20 (100%)	18.3±0.58 ^a (91.5%)	20 (100%)	0 (0%)
150	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	0 (0%)

Values with different superscripts differ significantly (P < 0.05)

The highest and the lowest concentrations of the *Sacoglottis gabonensis* extract which achieved 100% paralysis of the larvae at the most minimum time were 62.5 mg/ml and 15.625 mg/ml at 30 and 120 minutes respectively, as shown on Table 3.

DISCUSSION

The ethanolic extract of *Garcinia kola* seeds seems to exhibit selective anthelmintic activity. 100 mg/ml of the extract inhibited only 18.75% of the egg-hatch but paralysed 79.44% of the larvae. It is apparently more larvicidal than ovicidal. This may,

perhaps, be due to the different standard anthelmintics properties of both albendazole and levamisole, used as the positive controls. Such limited effects of different anthelmintics on different parasitic stages had been observed by Bogan and Armour, (1986). However, this contrasts sharply with the findings of Nwosu *et al*, (2004) which obtained 98.9% reduction in egg hatch with same concentration. The different solvent system (aqueous) used in their study may have been responsible for the disparity, (Sabir *et al*, 2007). The activity of the 50 mg/ml of the extract was compatible with the 12.5 mg/ml of the standard drug, levamisole, in paralysing 76.52% of the larvae, ($P > 0.05$). However, the efficacy of 25 mg/ml of the drug was significantly, twice, greater than that of the extract of same concentration which paralysed 47.04 % of the larvae. In all cases, the activity was concentration – dependent and all the observed paralysis were irreversible.

The methanolic extract of *Sacoglottis gabonensis* stem bark paralysed the larvae in a time and concentration – dependent manner. It took the maximum, (62.5 mg/ml) and the minimum, (15.625 mg/ml) concentrations of the extract 60 and 150 minutes respectively, to record 100% larval paralysis. However, only the activity of 125 mg/ml concentration of the extract was found to be compatible with 25 mg/ml of the standard drug, levamisole. Nonetheless, the findings here confirm the folkloric claims made for *Garcinia kola* seed and *Sacoglottis gabonensis* stem bark as traditional anthelmintics. Their exploration and exploitation could be encouraged for such a purpose, and incidentally, they are available and affordable especially in the tropical forest regions and also well tolerated in the body (Maduka and Okoye, 2003; Nwosu *et al*, 2001). However, *in vivo* toxicity studies need to be done with *Sacoglottis gabonensis* against which possible hepatotoxic activity had been reported (Udosen and Ojong, 1998). Only after such *in vivo* studies can the true efficacy of these plant extracts as anthelmintics be thoroughly established.

REFERENCE

- Asuzu, I. U. and Njoku, C.J., 1996. The anthelmintic effect of *Alstonia boonei* bark and *Nauclea latifolia* leaf aqueous extract on *Trichostrongylus* infective larvae. *Fitoterapia*, Vol. LXVII, No. 3: 220-222.
- Bogan, J. and Armour J., 1986. Anthelmintics for ruminants. Proceedings of the Sixth International Congress on Parasitology, 483-491.
- Chema, S. and Ward D., 1990. Cost effective disease control and animal health management in animal agriculture. Proceedings of FAO Expert Consultation held in Rome, Italy, Dec. 10 – 14th, 1990.
- Chiejina, S.N., Fakae B.B., Behnke J.M., Nnadi P.A., Musongong G.A. and Wakelin D., 2002. Expression of acquired immunity to a local isolate of *Haemonchus contortus* by the Nigerian West African Dwarf goat. *Veterinary Parasitology*, 104: 229-242
- Ebong, O.O. Karubo-Owiye O., 1996. West African Journal of Pharmacological Drug Research, 12:51-53.
- Fakae, B.B., Harrison L.J.S., Ross C.A. and Sewell, M.H., 1994. *Heligmosomoides polygyrus* and *Trypanosoma congolense* infections in mice - A laboratory model of concurrent gastrointestinal nematode and trypanosome infections. *Parasitology* 108: 61-68.
- Faparusi, S.I. and Osiyemi F.O., 1973. The biological effects of *Sacoglottis gabonensis* (Urban Humiriaceae) on micro organisms. *Experientia* 29: 634-635
- Jackson, F., 1993. Anthelmintic resistance – The state of play. *British Veterinary Journal*, 149:123 – 138.
- Keany, R.W.J., Onochie C.F.A. and Stanfield, D., 1964. Nigerian trees. Nigerian national Press Ltd. Dept. of Forest Research, Ibadan, Nigeria, p.184.
- Maduka, H. C. C. and Okoye, Z. S. C., 2003. The influence of *Sacoglottis gabonensis* extract and its isolate, bergenin, Nigerian alcoholic beverage additives, on the metabolic and haematological side effects of 2,4–dinitrophenyl hydrazine-induced tissue damage. *Vascular pharmacology* 39: 317-324.
- Martin, W.B. and Le Jambre, L. F., 1979. Larval paralysis as an *in vitro* assay of Levamisole and Morantel tartrate resistance in *Ostertagia*. *Veterinary Science Communications* 3: 159-164.
- Nwosu, C.O., Haruna N.K., Ogugbuaja V.O., Akinniyi A.O. and Onyeyili P. A., 2001. Anthelmintic efficacy of ethanolic stem bark extract of *Sacoglottis gabonensis* (Bail) against strongyline nematodes of small ruminants. *Journal of Life and Environmental Sciences*, 3: 127-130.
- Nwosu, C.O., Mobe, K.M., Gulani, I.G., Igbokwe, I.O. and Ogugbuaja, V.O., 2004. Anthelmintic efficacy of aqueous extracts of *Garcinia kola* seed and stem bark against strongylid nematodes of small ruminants in Nigeria. *Nigerian Journal of Parasitology* 25: 1-5.
- Prichard, R.K., 1994. Anthelmintic resistance. *Veterinary Parasitology*, 54:259-268.
- Sabir, M.S., Ahmad, D.S., Hussain, I.M. and Tahir, K.M., 2007. Antibacterial activity of *Elaeagnus umbellata* (Thunb.), a medicinal plant from Pakistan. *Saudi Medical Journal* 28 (2): 259-263.
- Udosen, E.O. and Ojong, A.S., 1998. Hepatotoxic activity of *Sacoglottis gabonensis* in rats. *Pharmaceutical Biology*, 3(5): p. 368-371