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Letter to Editor

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LARVICIDAL EFFECTS OF *AFRAMOMUM DANIELI* SEED EXTRACTS AGAINST GASTROINTESTINAL NEMATODE OF SHEEP: *IN VITRO* STUDIES

Key words: *Aframomum danielli*, larvicide, gastrointestinal nematodes, sheep

Dear Sir,

The most serious constraint affecting sheep production in Nigeria is gastro intestinal nematode parasitism, specifically *Haemonchus contortus*. Parasitic control of sheep over 30 years has been achieved almost exclusively by the use of proprietary anthelmintics and resistance of nematodes to these drenches has been reported worldwide (Craig and Miller 1990). The western developments in the elucidation and treatment of animal disease by-passed many livestock owners in poorer developing countries who continued to rely on their age-old methods in disease control and often considered western animal health care expensive, not embedded in local beliefs and concerns.

The seed of *A. danielli* are used as a traditional food spice among the Edo and Niger Delta people of Nigeria and also as an anti-inflammatory agent by rubbing of the alcohol and petrol extracts on the allergic and eczematous swelling. The fruit capsule of *A. danielli* is red and smooth, the seed are smooth, shiny, olive brown and may be almost flavourless or with a turpentine like taste and a variable pungency (Dalziel 1955).

Previous studies on *A. danielli* have shown the presence of diterpenes as acids and aldehydes. The aldehyde 8β - 17 epoxy – 12 E-labolane 15, 16 – dial exhibited potent antifungal properties. (Kimbu et al, 19794; Kim and Isoe 1983; Kindu, 1987). Water extracts of the rhizome also had strong molluscidal activity against *Bulinus globosus* snails implicated in schistosomiasis (Iwu 1993). However, there is no report in the literature on the anthelmintic activity of any *Aframomum species*.

In this paper, we report an assessment of the effect of *A. danielli* seed extracts on the survival of third stage larvae of strongyles of sheep.

The seeds of *A. danielli* (family: Zingiberaceae) were collected and unambiguously identified at the Department of Botany, University of Ibadan. The dried powered seeds were extracted with hexane and ethanol by continuous soxhlet extraction. While water extract was obtained simply by soaking fresh for about 24 hours. A stock solution of each extract was prepared in 10% propylene glycol solution. Dilution of appropriate aliquots of the stock solution was made to achieve the concentration of in the tubes ranging from 0.05 – 0.5 mg/ml.

The technique was that previously described by Hubert and Kerboeuf, 1992. Briefly, 10 – 15gm of faeces (from sheep predominantly infected with *Haemonchus contortus*) was collected per rectum and suspended in water and cleared of organic debris by titration through sieves, (1mm and 100 µm) the eggs been collected on a 20 µm sieve. The eggs were further cleared of organic debris by centrifugation in magnesium sulphate (density 1:10) for five minutes at 1000. The supernatant was filtered through 100 µm and 60µm sieves and the eggs were washed in water after which they were collected on a 20 µm sieve. The concentration of eggs was estimated in 50 µl samples and adjusted to 1200 to 1300/ml using.

The concentration of eggs was estimated in 50 µl and adjusted to 100 – 120 egg/ml. The egg suspension was diluted with the filtrate from the first step of egg extraction (described above) in order to provide rumen bacteria necessary for nematode larval development. To avoid the proliferation of fungi, 5 µg of amphotericin B was added per ml of egg suspension. The nutritive medium was described by Hebert and Kerboeuf, 1992

The test was carried out in 5ml tubes (diameter 11mm). 20 µl of nutritive medium was added to 80 µl of egg suspension containing approximately 100 eggs. Three replicates per extract concentration or water (control tubes) were made. The tubes were stoppered and put in an incubator at 27^0C for 48 hours by then the parasites had developed to first stage larvae then plant extract was added. The third stage larvae were obtained seven days later. At this time, the parasite were counted in a McMaster slide by separating the larvae into two classes, leaving third stage larvae (L_3)and dead larvae and living larvae of other stages.

Determination of 50 percent lethal concentration (LC_{50})

In larval survival assay, the LC_{50} is determined from the regression curve between the larvae development parameter expressed in probits and the extract concentration (mg/ml). The larval development parameter is given by

$$\frac{\text{Number of Living } L_3\text{/ Total number of nematode in wells (plant extract).}}{\text{Number of Living } L_3\text{/ Total number of nematode in control tube (water).}}$$

Data from LDA were transformed by probit transformation and plotted against the logarithm of concentration (Hubert and Kerboeuf 1992) probit transformation was performed to transform a typical sigmoid dose response curve to a linear function. The concentration required to kill 50 percent of L_3 (LC_{50}) was calculated from this linear regression scale

The crude ethanolic, hexane and aqueous extracts were subjected to qualitative phytochemical analysis using standard methods (Hamberg and Samuelsson 1974; Evans and Trease 1996).

The percentage yield of, ethanol, hexane, and aqueous extracts are 20.28 %, 7.61%, and10.39% respectively. The result of the phytochemical analysis revealed the presence of alkaloids, saponins, cardiac glycosides, steroids, and glycosides in the ethanolic extracts, alkaloids, cardiac glycosides, steroids and glycosides in the hexane extracts, while the aqueous extract contains alkaloids , saponins, glycosides, cardiac glycosides, and steroids. It was evident that *A. danielli* seed extract affect larval development following incubation period. A linear relationship was observed between survival of L_3 and *A. danielli* concentration (figure 1). The calculated LC_{50} of ethanolic, hexane and aqueous extract of *A. danielli* was 0.33 mg/ml, 0.39 mg/ml and 0.36 mg/ml respectively. The regression equations are $y = -4.4533x + 6.4847$; $y = -5.2097x + 7.0107$ and $y = -3.9612x + 6.4053$ respectively, while the R^2 are 0.9565, 0.9604, and 0.846 respectively.

A. danielli extract was shown by larval survival assay to exhibit anthelmintic activity. The hexane extract demonstrated a lower activity (LC_{50}, 0.39 mg/ml) compared with the ethanolic extract (LC_{50}, 0.033mg/ml). Phytochemical screening reveals the presence of saponins in the extract of *A. danielli.* The anthelmintic effect of *A. danielli* against nematode may be attributed to its saponins. Monodesmoside saponins destabilize membrane and increase cell permeability by combining with membrane associated sterols (Gee and Johnson1988; Monkiedje 1990). A loss of osmotic control leads to cytolysis and this property has been used in a haemolytic assay to assess the saponins content of the plant extracts (Kimbu et al, 1979). The extracts of *A. danielli* seed could find application in modern worm control in livestock by ethnoveterinary medicine approach.

Regular consumption of *A. danielli* in human diet could also reduce worm burden, especially in children.

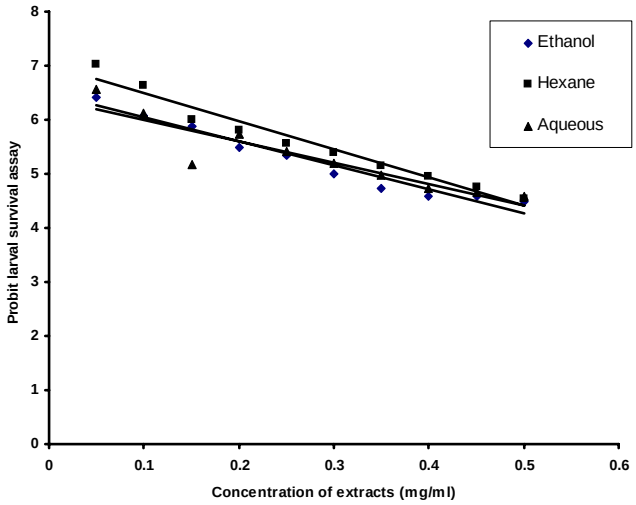

Figure 1: Linear relationship between mean values of L3 on the probit scale, of strongyles following a 7- day incubation period in *Aframomum danielli* extracts.

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