

## IN VITRO ANTHELMINTIC ACTIVITY OF *ANTHOCLEISTA DJALONENSIS*

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### SUMMARY

The *in vitro* anthelmintic activity of *Anthocleista djalonenensis* a folkloric medicinal plant was studied using the L<sub>3</sub> larvae of *Heligmosomoides polygyrus*. The ethanolic extract of *A. djalonenensis* (25, 50, 100, 200 mg/ml) exhibited a concentration-dependent lethal action on *H. polygyrus* larvae. After 6 h incubation the percentage mortality at 100 mg/ml was 98.45%, which was equivalent to that of levamisole (the positive control) at 10 mg/ml. At 25 and 50 mg/ml, the percentage mortality was 89.36%. After 24 h incubation the percentage mortality of *A. djalonenensis* extract was 41% at the highest concentration of 200 mg/ml, while levamisole at 10 mg/ml had 91%. Its LC<sub>50</sub> was 268.89+56.26 mg/ml using Minitab probit analysis. In conclusion, the ethanolic extract of *A. djalonenensis* exhibited a concentration-dependent lethal action on *H. polygyrus*.

**KEY WORDS:** *Anthocleista djalonenensis*, *Heligmosomoides polygyrus*, Extract, Levamisole

### INTRODUCTION

*Anthocleista djalonenensis* (family, Loganiaceae) is a medicinal tree in the folklore of the Ibo people in Nigeria. The decoction of the root is used to treat dogs, and such treated animals are claimed to pass out worms thereafter. Other ethnomedical uses include the treatment of diarrhoea and dysentery (Akubue et al., 1983). It is also used as an emmenagogue and abortifacient (Bouquet and Debray., 1974). It is used for intestinal pains and externally for furuncles and carbuncles (Le Grand, 1989), and for rheumatism (Akah and Nwambie., 1994).

The plant was found to be active against *Bacillus subtilis* at a concentration of 50 mg/ml (Le Grand et al., 1988). It induced intestinal motility in the ileum of guinea pig and mice (Akubue et al., 1983). The water extract showed weak molluscicidal activity at 100 ppm against *Bulinus globosus* (Okunji and Iwu., 1998). The ethanolic extract of *A. djalonenensis* had smooth muscle relaxant activity on guinea pig ileum and rabbit jejunum.

The LD50 of *A. djalonenensis* extract administered intraperitoneally was found to be 16 mg/kg in mice by Akubue et al., (1983). Antiinflammatory activity of the *A. djalonenensis* leaves and stem extract was observed in rat at 300 mg/kg (Akah and Nwambie., 1994). According to Akubue et al., (1983), it contains alkaloids and saponins. Onocha et al., (1995), found terpenes, steroids and carbohydrates while Okorie (1976) discovered the presence of lactone and alkanol (Bierer et al., 1995).

The aim of the present study was to investigate the *in vitro* anthelmintic efficacy of *A. djalonenensis* root ethanolic extract against the infective stage (L3) larvae of *Heligmosomoides polygyrus* a parasitic nematode of mice.

### MATERIALS AND METHODS

#### Plant material

*Anthocleista djalonenensis* root was collected in January, 2005 from Botany Department,

University of Nigeria, Nsukka and was authenticated by Mr. P. O. Ugwu. Voucher specimens were deposited in the departmental herbarium. The material harvested was air dried in a shade, pounded and pulverized to a fine dry powder in a laboratory mill (Thomas-Wiley laboratory mill, model 4, Arthur Thomas Co Philadelphia P.A. USA). It was kept in cellophane bags at room temperature until used.

#### Extract Preparation

The extract was prepared by cold maceration using 144.42 g of root powder in 2.5 L of 75% ethanol. It was left for 5 days with intermittent shaking. The suspension was filtered using Whatman No. 1 filter paper and allowed to evaporate to dryness on the bench. The extract was oily brown. The yield was 10.7% w/w.

#### Preparation of *Heligmosomoides polygyrus* L3 larvae

Faecal samples (10 g) were collected from Albino mice experimentally infected with *Heligmosomoides polygyrus*. A plastic tray containing a small amount of water was placed under the cage to wet the faecal samples in order to avoid desiccation. With the aid of mortar and pestle, the faecal samples were crushed and filtered using a coffee strainer. The filtrate was centrifuged at 1,800 rpm for 5 mins and the supernatant was discarded. The faecal samples were cultured by plating out the faeces on Petri dishes containing filter paper and kept in the refrigerator at 4°C where they were monitored daily. Small quantity of water was added to any Petri dish that was drying out. On the ninth day, the cultures were examined using a stereomicroscope (Stereozoom, Bausch and Lomb, Unified National Inventory Database). The L3 stage larvae were observed as they migrated out of the filter paper. Using small amount of water and a Pasteur pipette, the migrating larvae were harvested and put in a glass bottle, which was kept in the refrigerator at 4°C for use (Chiejina and Fakae, 1984).

#### Anthelmintic Evaluation

The extract was tested at doses of 12.5, 25, 50, 100 and 200 mg/ml, while levamisole served as the positive control at a concentration of 10 mg/ml. An average of 11 larvae/0.1 ml was put in each well of the microtitre plate and 0.1 ml of the extract or drug was added to give the above concentration. This was replicated 6 times. After 6 h and 24 h incubation at 40°C the larvae were examined at 40 times magnification and classified as normal if moving, or dead if no observable motion occurred during a five-second interval according to the method of Njoku and Asuzu, (1998).

Data were analysed using ANOVA and the LC50 calculated by Minitab probit analysis (Finney, 1971).

#### RESULTS

The LC50 was 268.89±56.26 mg/ml. After 6 h incubation the extract showed larvicidal activity against the L3 larvae of *Heligmosomoides polygyrus* at the concentrations tested. At 100 mg/ml of the extract, the percentage mortality recorded was 98.45% and was equal to that of levamisole at 10 mg/ml concentration (Table 1). The percentage death recorded for 50 and 25 mg/ml concentrations was 89.36%, while the untreated control had a percentage death of 37.82%.

After 24 h incubation (Table II), the percentage mortality at 200 mg/ml was 40.91%. At 100, 50 and 25 mg/ml, percentage mortalities were 31.82, 25.76 and 21.21%, respectively. Levamisole at 10 mg/ml had a percentage mortality of 90.91%, while that for the untreated negative control was 15.15%. There was a significant ( $P < 0.05$ ) difference between percentage mortalities obtained in groups A and C, also between groups A and E, but percentage mortality recorded in F was highly significantly ( $P < 0.01$ ) different from that of groups A and E respectively.

TABLE I: The effect of *Anthocleista djalonenis* root extract on *Heligmosomoides polygyrus* L3 larvae in vitro after 6 h

Groups	Concentration of extract/drug(mg/ml)	No of larvae in wells	Mean No of dead larvae	Percentage mortality
A	100	11	10.83 ± 0.17	98.45 ± 1.52 <sup>a</sup>
B	50	11	9.80 ± 0.48	89.36 ± 4.34 <sup>a</sup>
C	25	11	9.83 ± 0.54	89.36 ± 4.93 <sup>a</sup>
D	10 (Levamisole)	11	10.83 ± 0.17	98.45 ± 1.52 <sup>a</sup>
E	Untreated	11	3.67 ± 1.05	37.82 ± 9.58 <sup>b</sup>

Different superscripts on the percentage mortality column indicate significant difference between the means at the probability level: p<0.05

TABLE II: The effect of *Anthocleista djalonenis* root extract on *Heligmosomoides polygyrus* L<sub>3</sub> larvae in vitro after 24 h

Groups	Concentration of extract/drug(mg/ml)	No of larvae in wells	No of dead larvae in wells	Percentage mortality
A	200	11	4.50 ± 0.67	40.91 ± 6.1 <sup>a</sup>
B	100	11	3.33 ± 0.76	30.00 ± 6.52 <sup>ab</sup>
C	50	11	2.83 ± 0.48	25.45 ± 4.34 <sup>b</sup>
D	25	11	2.33 ± 0.42	20.91 ± 3.83 <sup>bc</sup>
E	10(Levamisole)	11	10.00 ± 0.37	90.91 ± 3.32 <sup>c</sup>
F	Untreated	11	1.67 ± 0.21	15.45 ± 1.92 <sup>a</sup>

<sup>bcd</sup> Different superscripts on the percentage mortality column indicate significant difference between the means: a b = a c = b d: p< 0.05; a d = c d; p< 0.01

## DISCUSSION

The ethanolic extract of *Anthocleista djalonenis* had larvicidal activity on the L<sub>3</sub> larvae of *Heligmosomoides polygyrus* at the concentrations tested. It, however, showed more activity at 6 h. The activity appeared to have decreased at 24 h. This suggests that the extract had a paralytic effect on the parasites, which decreased with time.

This is not new since the pharmacological basis of the treatment of helminthes could involve disruption of the energy processes. The mechanism of interference apparently occurred through reactions necessary for the generation of metabolic energy and subsequent paralysis of the parasite or neuromuscular coordination (depression of muscular activity), leading to paralysis of the parasite and their subsequent

expulsion (Bueding, 1969). Interference with the neuromuscular co-ordination in the parasite may occur by inhibiting the breakdown of excitatory neurotransmitters or by mimicking the action of excitatory neurotransmitters resulting in spastic paralysis of the parasite. Other mechanisms include mimicking the action of inhibitory neurotransmitters or causing hyperpolarization with an ensuing flaccid paralysis of the parasite (Clarence *et al.*, 1986). Either spastic or flaccid paralysis of an intestinal helminth allows the normal peristaltic actions of the host to expel the parasite. Examples of such drugs include levamisole and piperazine (Clarence *et al.*, 1986).

Immature and adult nematodes maintained in levamisole solution showed spastic

contractions followed by tonic paralysis. This is due to its cholinergic activity (Harrow and Gratton, 1985). This effect can be reversible or irreversible depending on the worm species, the concentration of the drug or the incubation conditions.

Piperazine and its derivatives are highly efficacious in ascariasis and oxyuriasis. According to Del Castillo *et al.* (1964), it acts by causing hyperpolarization in the membranes of the parasites. It causes muscular paralysis, which favours their expulsion by intestinal peristalsis, but does not kill ascarids. They are expelled alive and can become mobile again if placed in Ringer's solution at 37°C.

Although the mechanism of action of *A. djalonenensis* was not elucidated in the present study, it may be that the components responsible for the activity observed are apparently labile and decomposed over time in aqueous solution. The extract may also cause reversible paralysis of the nematodes, which may be responsible for the reduced effect observed over time. Further work needs to be done to ascertain the mode of action of *A. djalonenensis* and also the activity of the extract *in vivo*.

### CONCLUSION

In conclusion, the ethanolic extract of *Anthocleista djalonenensis* had larvicidal activity on the infective stage larvae of *Heligmosomoides polygyrus*. The activity observed was more after 6 h than after 24 h.

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