

# *In Vitro* Anthelmintic Activity of Leaf Extracts of *Celosia laxa* Schum. & Thonn

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## Abstract

**Background:** *Celosia laxa* (Amaranthaceae) is mainly a west African plant species used in Traditional Medicine as an anthelmintic, anticancer, antibacterial and analgesic agent. The seeming prevalence of worm infection in Gwandu community of Gwandu Local Government Area of Kebbi State-Nigeria prompted this study. **Methods:** Whole plant of *C. laxa* extracts obtained through maceration in aqueous (AE), methanol (ME) and hexane (HE) solvents were investigated for their anthelmintic activity against Indian earthworms (*Pheretima posthuman*) at four different (gradient) concentrations of 10, 20, 40 and 80 (mg/ml) for each extract. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. **Results:** Both the aqueous and ethanolic extracts exhibited significant anthelmintic activity at the highest concentration of 80 mg/ml as compared to the standard drug, praziquantel (10 mg/ml). Consequently, the aqueous extract showed a higher activity at 80 mg/ml compared to standard praziquantel at 10 mg/ml (with no significant value of  $p < 0.05$ ). The time of paralysis and death observed for AE was  $13.0 \pm 1.8$  and  $16.8 \pm 1.5$  while the ME was less bioactive with  $15.7 \pm 0.5$  and  $23.0 \pm 0.0$  respectively. However, on the other hand, the hexane extract recorded no-activity on all the test sample concentrations, compared to the standard drug (with a significant difference of p-value,  $p > 0.05$ ). **Conclusion:** It was concluded that the leaves of *C. laxa* are likely to yield a potent anthelmintic drug owing to soluble phyto-constituent which are largely hydrophilicity extracted by the polar solvents. Also, considering that the plants' mode of preparation for use by the locals was aqueous decoction before administration, the folkloric therapeutic claims can be said to have been justified.

## Keywords

Anthelmintic, Extracts, Maceration, Praziquantel, Phytoconstituents,

## 1. Introduction

Helminth is derived from the Greek word helminths—meaning, worm. Helminth is a broad categorical term referring to various types of parasitic worms that reside in the body [1]. The World Health Organization reveals that over two billion people are suffering from parasitic worm infections [2]. It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced [3]. Helminthic infections are very common in men. Helminthic infections can serve as a threat to human beings, especially in developing countries. It leads to malnutrition, anemia and pneumonia. Majority of the infections, which are due to worms are mostly limited to tropical regions. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminths or development forms that invade organs and tissues [4].

Celosia is a small genus of edible and ornamental plants belonging to the family Amaranthaceae. Among the different species in the genus celosia, *C. laxa* (Plate 1) is an important tropical leafy vegetable crop of high nutritional value [5]. An Indian origin of *C. laxa*, of tropical origin is known for its very brilliant colors with a wide range of traditional uses such as treatment of mouth sores, blood diseases, diabetes mellitus and as an aphrodisiac; with also claims of cure against ovarian and uterine diseases [6] [7]. However, *C. laxa* is largely a west African plant species used traditionally as an anthelmintic, anticancer, antibacterial and analgesic agent. The whole plant has also been reported for use in the treatment of diarrhea, piles, bleeding nose, as a disinfectant, inflammation, haematological and gynaecologic disorders [8].

Locally, the Hausas call it “bokan gida” or “nànnáfaá”, or “nànnàhoó”, while in Southwestern Nigeria it is called àjẹ fáwo. In west Africa of Sierra Leone and Gambia, it is called “gimbui” and “furarɲamo” respectively. The study was thus prompted by the fact that there was a seeming prevalence of worm infection in Gwandu community of Gwandu Local Government Area of Kebbi State-Nigeria.

Earlier interviews among the locals in the various village communities of Gwandu LGA in Kebbi State-Nigeria, revealed several cases and complains of stomach ache and parasitic diseases leading to the use of a decocted plant species of *C. laxa* to effect cure. Thus, the seeming prevalence of worm infection and diseases that bore similarities with the later in these communities prompted this study. Moreover, it has been reported that most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea [9].

Hence, Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections [10]. Literature has also shown that increasing problems of development of resistance in helminths against anthelmin-

tics have led to the proposal of screening medicinal plants for their anthelmintic activity [11]. Consequently, based largely on folklore therapeutic claims, the present study was carried out to assess the anthelmintic activity of *Celosia laxa* against *Pheretima posthuma* using three extracts obtained from solvents of gradient polarity.

## 2. Materials and Methods

### 2.1. Plant Collection and Authentication

Leaves and whole plant of *Celosia laxa* were collected in April 2017 from Gwandu town of Kebbi State, Nigeria. It was authenticated using compendium by Rogers of the Hausa Plant-Scientific names compilations. Herbarium specimen was thereafter prepared and deposited at the Department of Pharmacognosy, Usmanu Danfodiyo University Sokoto (UDUS) with voucher number PCG/UD-US/Amar/0005.

### 2.2. Worms Collection and Authentication

*Pheretima posthuma* was collected from the water-logged areas of the soil and identified and authenticated at the Veterinary Faculty of UDUS.

## 3. Preparation of Extract

### 3.1. Aqueous, Methanol and Hexane Extract Preparation

The crude aqueous extract of *C. laxa* leaves was prepared according to the standard method. One hundred grams of the powdered plant material was mixed with 500 mL of distilled water in a Soxhlet apparatus for 8 - 12 h. The filtrate was concentrated in a rotary evaporator and the extract labeled AE, was stored at 4°C until required. Methanol extract was obtained from the dried powder (350 g) by maceration for two days in 1.7 liters of 70% methanol solvent. The extract was evaporated in vacuo to obtain a dark green residue and labeled ME. This was stored at 4°C until use. Similarly, 350 g of powdered *C. laxa* plant were macerated in n-hexane solvent of 750 mL for some 18 hours, filtered and concentrated to obtain a greenish extract labeled, HE. This was stored at 4°C until use.

### 3.2. Phytochemical Evaluation

The qualitative chemical tests to detect the various phytoconstituents were carried on the extracts of *C. laxa* plant using the methods described by [12] [13] [14].

### 3.3. Anthelmintic Assay

The method of [15] was adopted with slight modifications. Adult earthworm (*Pheretima posthuma*) of uniform size was collected at water logged areas, were identified, and washed with distilled water to remove dirty matter. The worms were divided into four groups, each containing six worms. The plant extracts and standards (10 mg/mL) were poured into petri-dishes and the earthworm

released. For the extracts, 100 ml formulations containing four different concentrations (10, 20, 40 and 80) mg/ml in distilled water, each of aqueous, ethanol and hexane extract, were prepared and the six worms (of same type) were placed in it.

Distilled water was used in place of plant extract for the control group. All solutions were prepared freshly before starting the assay.

Movement of the worms were monitored in terms of changes in rapid movement, the release of body exudates, segment breakage and decolorization; by observing keenly with the naked eye as well as with the aid of a hand lens magnifier. Time was noted for the death of worms, which was confirmed by immobility and fading of body colour of worms. Also, paralysis was noted by dipping non-motile worms in hot water to see for possible resurrection. Generally, time for paralysis as well as death of the worms were noted and recorded.

### 3.4. Data Analysis

The result of each group of experimental animals were express as mean (%)  $\pm$  standard error of mean (S. E. M.). Data were analyzed using one-way factorial ANOVA tests, followed by Dunnett's *t*-tests on each group. P values under 0.05 were considered highly significant (shown as \*\*).

## 4. Results

### Preliminary Phyto-Constituent Analysis

Phytochemical screening of the extracts revealed richly, the presence of alkaloids, cardiac glycosides and less richly some other metabolites which are mainly polyphenolics. These are shown in **Table 1**. While, chemo-microscopical studies carried out on the powdered drug of *C. laxa* revealed the presence of fat and oil, calcium oxalate and starch as shown in **Table 2**.

## 5. Discussions

All anthelmintics essentially kill worms by either starving them to death or paralyzing them because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs [17]. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hours or less is sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut [18]. Preliminary phytochemical screening of *C. laxa* extract recorded on **Table 1** revealed the presence of proteins, saponins, steroids, carbohydrates, alkaloids, tannins, glycosides, flavonoids and phenols. Phytochemical qualitative test performed from earlier study of same plant conformed with this result [19].

Phenolic anthelmintics interfere with the energy generation in the helminth parasites by uncoupling the oxidative phosphorylation. Another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death.

**Table 1.** Phytochemical screening of *Celosia laxa* leaves extract.

Test	Observation	Inference
<b><u>Simple sugar</u></b>		
a) Molish's test	Purple colour observed at the interface	+
b) Fehling's test	Brick red precipitate formed	+
<b><u>Alkaloids</u></b>		
a) Mayer's test	Cream precipitate formed	++
b) Dragendoff's test	Reddish brown precipitate formed	++
c) Wagner's test	Reddish brown precipitate	++
<b><u>Saponin</u></b>		
Frothing test	Frothing formed	+
<b><u>Cardiac glycosides</u></b>		
a) Keller-killani's test	Brown ring colour is formed	+
b) Salkowski's	Reddish-brown colour at the interface formed	++
<b><u>Polyphenol</u></b>		
Ferric chloride	Colour change observed	+
<b><u>Tanins</u></b>		
Ferric chloride	Brownish green precipitate	+

Key: + = detected; ++ = highly detected; -- = not detected

**Table 2.** Chemo-microscopical observation of *Celosia laxa*.

Test	Observation	Inferences
Calcium oxalate	Calcium oxalate crystal was observed	Calcium oxalate present
Fat and oil	Reddish colouration was observed	Fat is present
Starch	Blue black colouration observed	Starch present

The possible mechanism of action of tannins may be three-fold as follows: 1) interfere with energy generation by uncoupling oxidative phosphorylation; 2) they may interfere with glycoprotein of cell surface and; 3) they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [20] [21].

Alkaloids may act on central nervous system and caused paralysis of the earthworm [18] [22]. The effect would be due to presence of the steroidal alkaloid, oligoglycosides, which may suppress the transfer of sucrose from the stomach to the small intestine; hence, couple with its antioxidant effect, it is capable of reducing the nitrate generation which could interfere in local homeostasis that is essential for the development of helminths [23].

Praziquantel works by causing severe spasm and paralysis of the worms' muscles, accompanied by  $\text{Ca}^{2+}$  influx inside the schistosome. Morphology alteration

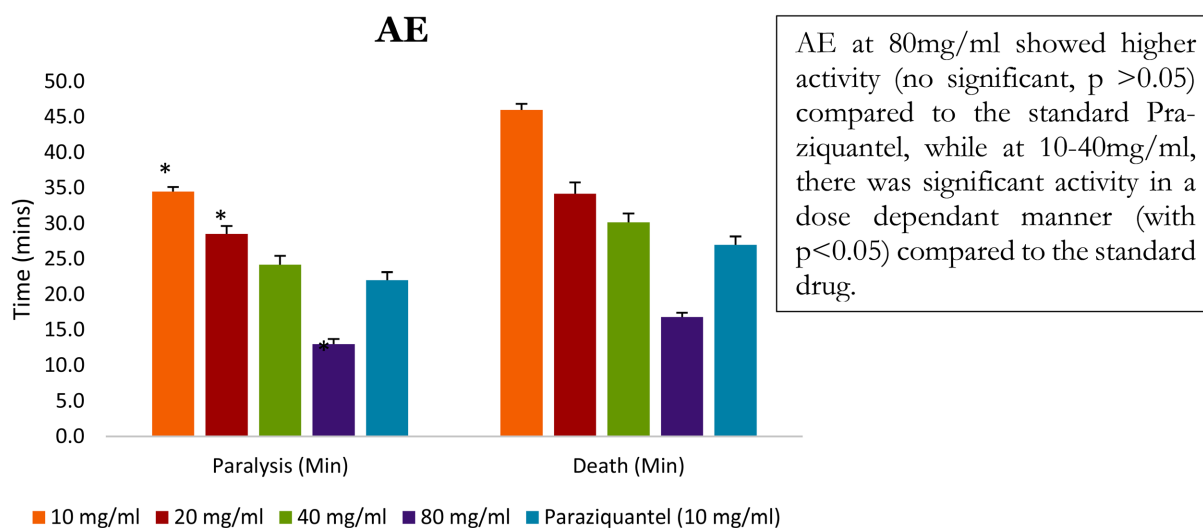
and a host of other factors are the mode of actions of the standard drug used [24]. Thus, the ergastic cell content recorded in the chemo-microscopy of *C. laxa* (Table 2), may play some role in the anthelmintic potency observed by the plant's hydroalcoholic extracts.

Analyzing results given in Table 3 and the graphs (Figure 1 and Figure 2) showed that the aqueous extract, AE at 80 mg/ml gives higher activity (no significant,  $p > 0.05$ ) compared to the standard Praziquantel, while at 10 - 40 mg/ml, there was significant activity in a dose dependant manner (with  $p < 0.05$ ) compared to the standard drug. Hence, this shows that the aqueous extract, AE, as seen in the graph (Figure 1), exhibited the most anthelmintic activity with the least time required to paralyzed and completely kill the earthworms at every tested concentration. Anthelmintic potency of the aqueous extract was closely followed by the methanolic extract, ME, as shown in the graph (Figure 2), which also exhibited significant anthelmintic activity in a dose dependent manner. However, in a conversely revealed activity pattern of AE and ME, the

**Table 3.** *In vitro* evaluation of anthelmintic activity of *C. laxa* leaves

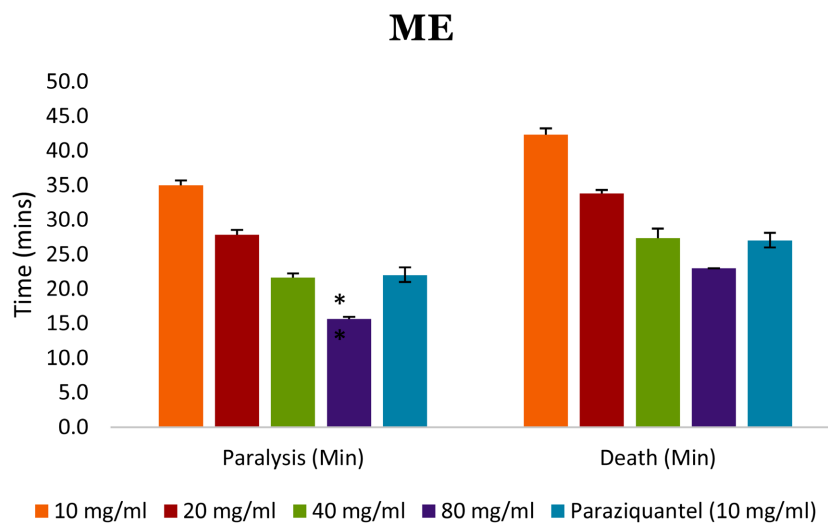
Treatment (Extract)	Paralysis Time in Mins (Mean $\pm$ SD)	Death Time in Mins (Mean $\pm$ SD)
<b>Aqueous</b>		
10 mg/ml	34.5 $\pm$ 1.5*	46.0 $\pm$ 2.1
20 mg/ml	28.5 $\pm$ 2.7*	34.2 $\pm$ 4.0
40 mg/ml	24.2 $\pm$ 3.1a	30.2 $\pm$ 3.1
80 mg/ml	13.0 $\pm$ 1.8**	16.8 $\pm$ 1.5
Praziquantel (10 mg/ml)	22.0 $\pm$ 2.0c	27.0 $\pm$ 2.0
<b>Methanol</b>		
10 mg/ml	35.0 $\pm$ 1.8	42.3 $\pm$ 2.3
20 mg/ml	27.8 $\pm$ 1.7	33.8 $\pm$ 1.2
40 mg/ml	21.7 $\pm$ 1.5	27.3 $\pm$ 3.4
80 mg/ml	15.7 $\pm$ 0.5	23.0 $\pm$ 0.0
Praziquantel (10 mg/ml)	22.0 $\pm$ 2.0 c	27.0 $\pm$ 2.0
<b>Hexane</b>		
10 mg/ml	10.6 $\pm$ 0.8	23.8 $\pm$ 1.1
20 mg/ml	13.0 $\pm$ 2.0	23.0 $\pm$ 2.0
40 mg/ml	35.0 $\pm$ 2.0	45.3 $\pm$ 0.6
80 mg/ml	22.0 $\pm$ 2.6	27.0 $\pm$ 1.0
Praziquantel (10 mg/ml)	22.0 $\pm$ 2.0 c	27.0 $\pm$ 2.0

Values are expressed as mean  $\pm$  SEM. Values were found out by using ONE way ANOVA followed by Dunnett's *t*-test. \*\* Values are significantly different from control at ( $P < 0.05$ ).



Where, AE = aqueous extract of *C. laxa*. Values are expressed as mean  $\pm$  SEM. Values were found out by using ONE way ANOVA followed by Dunnett's *t*-test. \*\*Values are significantly different from control at ( $P < 0.05$ ).

**Figure 1.** Time taken for paralysis of *C. laxa* by AE as compared to Praziquantel.



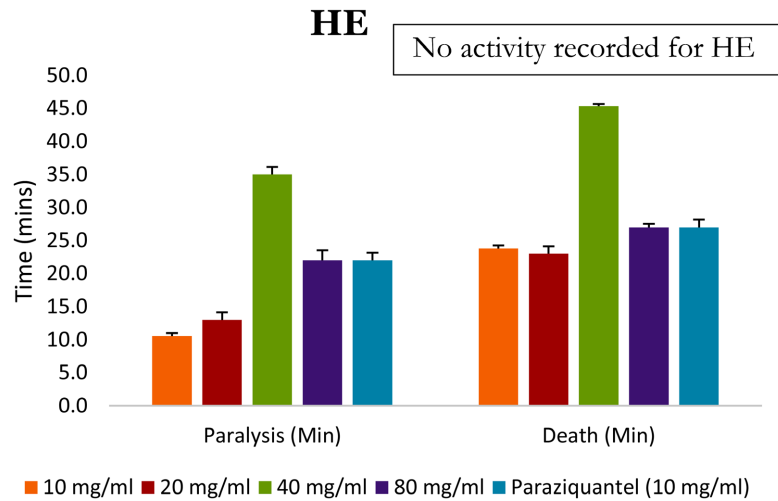
Where, ME = ethanolic extract of *C. laxa*. Values were found out by using ONE way ANOVA followed by Dunnett's *t*-test. \*\*Values are significantly different from control at ( $P < 0.05$ ).

**Figure 2.** Time taken for paralysis of *C. laxa* by ME as compared to Praziquantel.

hexane extract, HE, showed no significant activity, as seen from the graph (**Figure 3**). These are not far-fetched, as most research groups have estimated that greater amounts of polyphenolics are resident in polar hydroalcoholic domains.

## 6. Conclusion

The overall findings of the present study have shown that *C. laxa* contains possible anthelmintic compounds due to the bioactivity records of aqueous and methanol extracts. Extract from hexane solvent had recorded no activity at the concentrations tested, indicating a likely non-synergistic mode of action by the



Where, HE = hexane extract of *C. laxa*. Values are expressed as mean  $\pm$  SEM. Values were found out by using ONE way ANOVA followed by Dunnett's *t*-test. \*\*Values are significantly different from control at ( $P < 0.05$ ).

**Figure 3.** Time taken for paralysis of *C. laxa* by HE as compared to Praziquantel.



**Plate 1.** *Celosia laxa* thriving gracefully in cultivated land habitat (Image retrieved [16]).

phytoconstituents in the three extracts. Alkaloids and cardiac glycosides were detected richly in the plant and hence, not ruling out their role in the bioactivity recorded. The *in vitro* method used has thus, provided a means of rapidly screening the plant's extracts for the validation of its claims as an anthelmintic and hence, indicate the plant's potentials. Further studies, using the results of these



findings as a lead, might just rightly translate to the discovery of more potent anti-infectives against parasitic (helminth) worm disease.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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