



In vitro* anthelmintic activity of the seed extracts of three plants of the Cucurbitaceae family on *Lumbricus terrestris

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Abstract

Aqueous and methanolic extracts of the seeds of *Cucurbita pepo*, *Citrullus lanatus* and *Telfairia occidentalis* (all of the family: Cucurbitaceae) were evaluated *in vitro* for anthelmintic activity against *Lumbricus terrestris* (earthworms). Seven motile worms were released into solutions of various concentrations of extracts (2.5, 5, 10, 20, and 50mg/ml in normal saline). Albendazole (1, 2.5, 5 and 10mg/ml) and normal saline served as positive and negative controls respectively. Results were expressed in terms of time for paralysis and death of the worms. The aqueous and methanolic extracts, at all the doses tested, caused concentration-dependent paralysis and death of the worms. *Cucurbita pepo* was found to be most effective in killing the worms compared to *C. lanatus* and *T. occidentalis* extracts. Also, the aqueous extracts showed higher worm inhibitory and destructive activities compared to the methanolic extracts. The results show that *Cucurbita pepo*, *Citrullus lanatus* and *Telfairia occidentalis* possess potent anthelmintic activities, thus supporting their use in traditional medicine for management of helminth infections.

Keywords: Helminth, *Telfairia occidentalis*, *Cucurbita pepo*, *Citrullus lanatus*, aqueous extracts

INTRODUCTION

Helminth infections are among the most widespread infections in humans which affect a large percentage of the world population. They are also a major constraint in successful productivity of livestock industries. Majority of these infections are generally restricted to tropical regions and cause enormous hazard to health contributing to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia (Bundy 1994). The emergence of anthelmintic resistance against virtually all available commercial drugs has posed serious impediments to the effective control of helminth infestations (Sondhi *et al.*, 1994;

Waller and Thamsborg, 2004), hence the increasing demand towards natural anthelmintics and tendency towards medicinal plants as alternative anthelmintics.

Medicinal plants have served through the ages, as a constant source of medicaments for the exposure of variety of diseases and helminth infestations are not an exception. Various plants that have been studied for their anthelmintic properties include *Acanthus montanus*, *Ananas comosus* (Okujagu *et al.*, 2006), *Heliotropium indicum* (Assiak *et al.*, 2001), and *Allium sativum* (Ajaiyeoba *et al.*, 2003). *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Watermelon) is a vine-like flowering plant and contains about 6% sugars and 92%

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water by weight (Dane and Liu, 2007). As with many other fruits, it is a source of vitamin C. The seed is demulcent, diuretic and tonic and is sometimes used in the treatment of the urinary passages and bed wetting. The seed is also a good vermifuge and has hypotensive actions. The fruit, when eaten fully ripe is used as a febrifuge and diuretic, being effective in the treatment of dropsy and renal stones. The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes while the root is purgative, and in large doses, is said to have emetic properties.

Cucurbita pepo (pumpkin or gourd) also variously known locally as “*Elegede*”, “*Ugbogolu*”, or “*gboro*” has been much used as medicine in Central and North America. It is a gentle and safe remedy for a number of complaints, especially as an effective tapeworm remover for children and pregnant women for whom stronger acting and toxic remedies are unsuitable. The seeds have been used as anthelmintic and taenicide, demulcent, diuretic and tonic (Chung and Ko, 1976, Zhang *et al.*, 1994). A tea made from the seeds is used as a remedy for hypertrophy of the prostate gland (Mahmoud *et al.*, 2002) and in the treatment of nephritis and other problems of the urinary system (Dreikon, 2002).

Cucurbita pepo leaves are applied externally to burns while the fruit pulp is used as a decoction to relieve intestinal inflammation. The presence of polyunsaturated fatty acids tocopherol and carotenoids in *C. pepo* has made it valuable for use as an adjuvant in the treatment of diet related hyperlipoproteinaemia, as well as in the prophylaxis of atherosclerosis. *Telfairia occidentalis* (fluted pumpkin), locally called Ugu, among the Igbos of South East Nigeria, is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. The young leaves sliced and mixed with coconut water and salt are used for the treatment of

convulsions (Gbile, 1986) while the roots are used as rodenticide and an ordeal poison (Gill 1992).

Telfairia occidentalis leaves have been reported to possess antioxidant and hepatoprotective (Oboh and Akindahunsi, 2004; Nwanna and Oboh, 2007), antiplasmodial (Okokon *et al.*, 2007), antianaemic (Alada, 2000, Dina *et al.*, 2006), antimicrobial (Oboh *et al.*, 2006) and hypoglycemic (Aderibigbe *et al.*, 1999; Eseyin *et al.*, 2005) properties as well as to protect and ameliorate brain and liver damage induced by malnutrition in rats (Kayode *et al.*, 2009, 2010).

Various unsubstantiated claims have been made regarding the anthelmintic properties of the Cucurbitaceae family. In the present study, we have investigated the possible anthelmintic activity of medicinal plants of the Cucurbitaceae family, namely *Citrullus lanatus*, *Cucurbita pepo* and *Telfairia occidentalis* which are part of everyday diet of the local populace. Also, an attempt has been made to compare and evaluate the anthelmintic potencies of the aqueous and methanolic seed extracts of these plants

EXPERIMENTAL

Plant materials. The seeds of *Telfairia occidentalis*, *Citrullus lanatus* and *Cucurbita pepo* were collected from the local market in Benin City, in the months of July and August, 2011. The plant materials were authenticated by Dr. B. A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, where voucher samples have been deposited for future reference. The seeds were cleaned, washed and shade-dried. The dried materials were ground to coarse powder using a mechanical grinder.

Preparation of extracts. Powdered seeds (500g) of *C. pepo*, *T. occidentalis* and *Citrullus lanatus* were each used for the aqueous and methanolic extraction. The

aqueous extracts were obtained by maceration in 1L of distilled water for 24 hours at room temperature while the methanolic extracts were obtained by maceration of the powdered seeds in 1L of methanol for forty eight hours at room temperature after which they were filtered using a muslin cloth. The filtrates obtained from both aqueous and methanolic extractions were concentrated *in vacuo*. The extracts were placed in air tight bottles separately and stored in the refrigerator for further use.

Tests were carried out on the powdered samples of the plant materials to determine the phytochemical constituents using standard procedures (Evans, 1989).

Animals. Adult Nigerian earthworms (*Lumbricus terrestris*) collected from moist areas in Benin City, Nigeria were identified by Dr. Chris Oke of the Department of Animal Science and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The earthworms (7.5 ± 0.65 cm in length and 0.2 ± 0.05 cm in width) were washed with normal saline to remove dirt and faecal matter.

Anthelmintic activity. *In vitro* method of investigating anthelmintic activity of medicinal plants was used (Ajaiyeoba *et al.*, 2001). The aqueous and methanolic extracts of *T. occidentalis*, *C. pepo* and *C. lanatus* were prepared at 2.5, 5, 10, 20 and 50mg/ml in normal saline. Seven earthworms were placed in each Petri dish containing 30ml of above test solutions of extracts or Albendazole (1, 2.5, 5 and 10mg/ml). Normal saline was used as the control. All the test solutions and standard drug solutions were freshly prepared prior to the experiments. The time taken for paralysis (cessation of movement of any sort which did not revive in normal saline) and death (complete loss of motility followed with fading away of body colours) to occur was recorded. The death of

worms was further confirmed by transferring the motionless worms to warm water at 40°C (Matthew *et al.*, 1995; Dash *et al.*, 2003), which stimulates and induces movement in the worms, if alive.

Statistical analysis. Results are expressed as mean \pm SEM. Statistical analysis of the data was done using Student's t-test and the results were considered significant when $P < 0.05$

RESULTS

Preliminary phytochemical analyses of the plant materials revealed the presence of carbohydrates, tannins, saponins, reducing sugars and flavonoids.

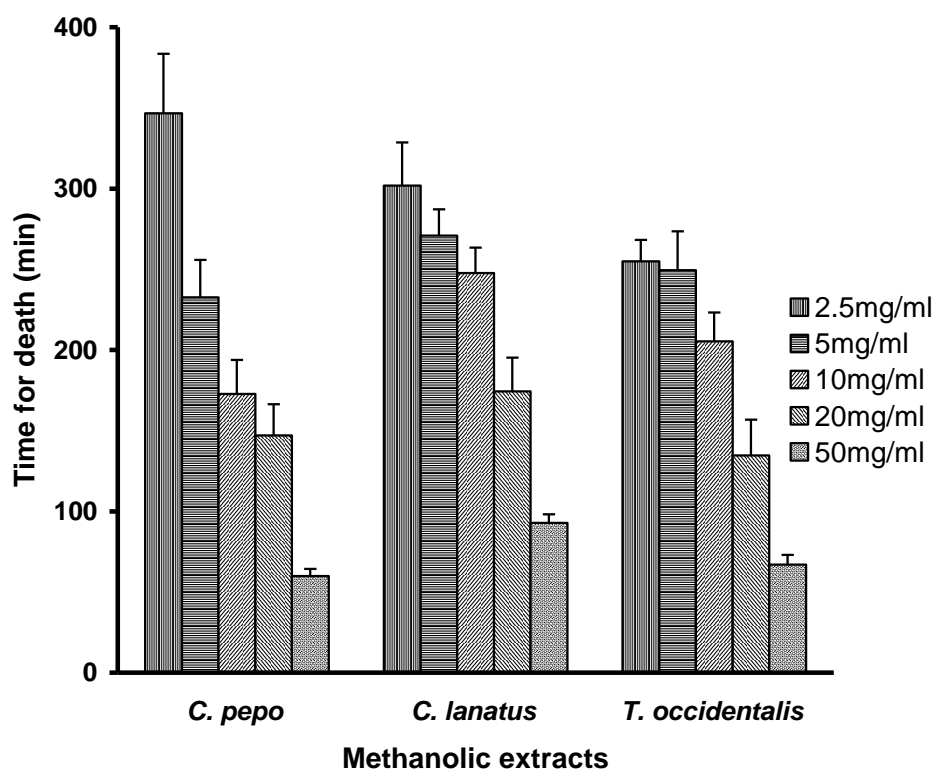
Aqueous and methanolic extracts of *Cucurbita pepo*, *Citrullus lanatus* and *Telfairia occidentalis* caused significant ($p < 0.001$) and concentration-dependent paralysis and death of the worms. All the extracts showed pronounced anthelmintic activity at the lowest concentration (2.5 mg/ml) tested (Table 1 and Fig. 1). Both aqueous and methanolic extracts of *C. pepo* showed the highest activity compared to *C. lanatus* or *T. occidentalis*. Generally, in all the plants tested, the aqueous extracts showed greater anthelmintic activity than the methanolic extracts.

Albendazole (10mg/kg) exhibited anthelmintic activity which was significantly higher than those of the highest concentrations of *C. lanatus* and *T. occidentalis*. However, the effect of *C. pepo* at 50mg/ml, in paralysis and death of the worms, was comparable to Albendazole (Table 1).

Table 1: Anthelmintic activity of aqueous and methanolic extracts of *C. pepo*, *C. lanatus*, *T. occidentalis* and Albendazole on *Lumbricus terrestris*

Group	Concentration (mg/ml)	Aqueous extract	
		Paralysis time (min)	Death time (min)
†Control (Normal saline)	-	-	-
<i>C. pepo</i>	2.5	196.19±12.23*	290.39±23.36*
	5	138.71±12.96*	201.04±22.6*
	10	99.17±9.67* ^a	186.14±27.84* ^a
	20	55.72±3.03* ^a	85.27±3.58* ^a
	50	37.25±1.60* ^a	50.49±2.28*
<i>C. lanatus</i>	2.5	179.43±17.35*	249.74±19.13*
	5	175.77±12.74*	227.08±13.28*
	10	116.97±16.35* ^a	154.64±17.59* ^a
	20	76.59±12.22* ^a	112.84±18.19* ^a
	50	38.49±1.20* ^a	58.2±3.41* ^a
<i>T. occidentalis</i>	2.5	201.08±20.35*	248.35±22.90*
	5	181.04±12.13*	235.56±13.86*
	10	157.96±10.91* ^a	197.11±15.07* ^a
	20	103.95±11.67* ^a	158.61±9.41* ^a
	50	42.97±1.45* ^a	66.63±4.10* ^a
Albendazole	1	138.00±31.35*	215.14±21.16*
	2.5	81.11±17.10*	154.00±13.18*
	5	34.43±0.43*	62.62±1.52*
	10	28.68±1.49*	51.91±1.86*

Values are mean ± SEM (n = 7). *p<0.001 compared to control. ^ap<0.05 compared to Albendazole (10mg/ml).
 †Control animals alive for over 12 hours

**Figure 1.** Comparative anthelmintic effects of the methanolic extracts of *C. pepo*, *C. lanatus* and *T. occidentalis* on *Lumbricus terrestris*

DISCUSSION

In this study, the anthelmintic activities of *Citrullus lanatus*, *Cucurbita pepo*, and *Telfairia occidentalis* were evaluated using an *in vitro* method in which earthworms are the test organisms.

This model has been widely used to assess the anthelmintic properties of plants (Ajaiyeoba *et al.*, 2001) since the earthworm resembles, anatomically and physiologically, the intestinal round worm parasites of the human beings in addition to their easy availability and accessibility (Dash *et al.*, 2002; Nirmal *et al.*, 2007).

Both the aqueous and methanolic extracts of the plants demonstrated concentration-dependent anthelmintic activities with maximal effect seen at a concentration of 50 mg/ml. *C. pepo* had the fastest anthelmintic action (death time of 50 and 60 minutes for aqueous and methanol extracts, respectively), effects similar to that produced by albendazole, a benzimidazole anthelmintic which acts by high affinity binding of parasite β -tubulin and preventing the incorporation into microtubules essential for energy metabolism (Borgers and De Nollin, 1975, Lacey, 1990).

Phytochemical analyses of the extracts revealed the presence of carbohydrates, tannins, saponins, reducing sugars and flavonoids. Tannins, which are polyphenolic compounds, are known to produce anthelmintic effects through interference with energy generation in helminth parasites and uncoupling of oxidative phosphorylation (Martin, 1997), or binding of glycoproteins on the cuticle of the parasite (Thompson and Geary, 1995) thereby leading to death. Since the extracts contain polyphenolic compounds, it is possible that their anthelmintic effects may result from this mechanism.

Also, plants belonging to the genus, *Cucurbita* have been found to contain cucurbitin. This active amino acid has been

shown to be a potent anthelmintic agent which eliminates intestinal parasites, including tapeworms and roundworms, by forcing them to loosen their grip and get excreted from the body (Mihranian *et al.*, 1968).

The differences in the anthelmintic effects observed with the different extracts may be attributed to the different abilities of the active constituent(s) present in the various plants to permeate the lipid cell membrane of *Lumbricus terrestris*, which is rich in sterols especially ergosterol.

In conclusion, the study showed that the aqueous and methanolic extracts of *Cucurbita pepo*, *Citrullus lanatus* and *Telfairia occidentalis* possess significant and concentration-dependent anthelmintic activities against roundworms. There was an inverse relationship between their potencies and the time taken for paralysis and/or death of the worms to occur.

Cucurbita pepo was found to be most effective in killing the worms compared to *C. lanatus* and *T. occidentalis* extracts. Also, the aqueous extracts exhibited higher inhibitory and killing effects on the worms compared to the methanol extracts.

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