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## Osmotic Effect of Terminalia Catappa Linn. Leaf Extract on Adult Onchocerca Volvulus by Modified in-Vitro Bioassay, Using the Parameter of Weight

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### Abstract:

**Background:** *Onchocerca volvulus* is a causative agent of Onchocerciasis, a leading cause of blindness. This study was to determine the osmotic effect of *Terminalia catappa* (Tropical Almond) leaf extract on the adult *Onchocerca volvulus* by modified in-vitro bioassay, using the parameter of weight.

**Method:** A total of 252 adult worms (*Onchocerca volvulus*) sourced via nodulectomy were used for the study. The crushed leaves of *Terminalia catappa* was extracted using 95% ethanol, filtered, and evaporated (Tabassam method). The dried extracts were dissolved in sterile dilutions to prepare dose concentrations of 10mg/ml, 20mg/ml and 40mg/ml. Seventeen labeled laboratory beakers were used for the study. At room temperature (25-30°C), six life worms per beaker were individually weighed, using electronic weighing instrument (OHAUS) and weights recorded before they were introduced into the different doses of *Terminalia catappa* leaf extract. The worms were observed for paralysis and death. Upon death, the worms were again weighed and results recorded. A positive control beaker contained anthelmintic drug Albendazole and a negative control beaker, contained saline water. The procedure was repeated three times.

**Result:** The methanolic *Terminalia catappa* leaf extracts effect on *onchocerca volvulus* recorded the following mean average results upon the death of worms. The concentration dose of 40mg/ml leaf extract showed  $0.005 \pm 0.001$  weight loss. The dose of 20mg/ml and 10mg/ml leaf extract recorded  $0.003 \pm 0.001$  and  $0.002 \pm 0.000$  weight loss respectively. The reference drug (Albendazole) showed a reduction in the mean average weight of worms upon death  $0.005 \pm 0.006$  compared to the live weight. And worms in Saline water recorded a weight increase of  $.001 \pm 0.006$  upon dead.

**Conclusion:** This weight reduction was observed as a consistent pattern with the methanolic *Terminalia catappa* leaf extract and therefore possesses concentration dependent osmotic mechanism on *Onchocerca volvulus*. With saline water, the worms rather increased in weight upon death.

**Keywords:** *Terminalia catappa*, *Onchocerca volvulus*, nodulectomy, Albendazole

### 1. Introduction

Onchocerciasis is recognized as a major deleterious disease of massive public health and socioeconomic concern [21]. It is classified as a Neglected Tropical Disease by the World Health Organization (WHO), with hundreds of thousands of people blind and an increasing number of individuals at risk of more infection [6]. Empirical findings revealed that it is the second leading cause of blindness caused by infection with the *Onchocerca volvulus* nematode [6]. Onchocerciasis is a severe public health concern has ravaged the *Simulium damnosum* (black fly) endemic local communities of South Eastern Nigeria [1] About 99% of cases are found in Africa where 85million people live in endemic areas [2]. Nigeria has the highest number of persons with onchocerciasis, accounting for over one-third of the global prevalence [3]. According to the records of the African Program on Onchocerciasis Control (APOC), 508 of 779 Local Government Areas in Nigeria have indicators of serious onchocerciasis infection that about 35,210 communities are endemic with onchocerciasis and on the whole, more than 25 million Nigerians are at risk of infection. The Nigerian Federal Ministry of Health surveyed in 1993 - 1994 to determine the level of endemicity nationwide; Imo State in the southeastern region of Nigerian was found to be highly endemic with over 1.1 million persons at risk of getting infected with onchocerciasis [4].

This grim prospect stimulated the need for this research work on *Terminalia catappa* leaf extract and which can represent a major opportunity to discover a new lead therapeutic module with anthelmintic potential, and which are also sustainable and environmentally friendly [5][6]. The history of the medicinal purposes of plants is probably as old as mankind. Herbs have medicinal property due to the presence of different active constituents like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols. Medicinal plants are continuously revolutionizing the face of the earth through all the distinctive benefits they render [7]. The World Health Organization (WHO) observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It is also estimated that 4 billion people (80% of the world population) presently use herbal medicine for health-care purposes [2]. Most of these plants contain potent active compounds that inhibit and cure several ailments. This has led to the increasing

global demand for these nature's gifts [7]. Even though most of these plants are very close to us, yet it is so appalling that most of us fail to recognize and exploit them [8].

*Terminalia catappa* (Linn) has been investigated in various pharmaceutical studies as it contains a variety of chemical components [9]. The plant extracts exhibit anthelmintic as well as biological activities, including antioxidant (punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, cumaric, and its derivatives) [10]. It also contains anti-inflammatory (triterpenic acids, especially ursolic acid and its derivatives) [11], antimicrobial (flavones and flavanols) [12] and hepato-protective activities (punicalagin, punicalin), [13]. In India, a plaster of *T. catappa* leaves is used to treat scabies, leprosy wounds and other skin diseases [12]. Its traditional uses especially in India, the Philippines and Malaysia include the treatment of diarrhoea and fever [13].

## 2. Collection and Extraction of Leaf

*Terminalia catappa* leaves were collected from the plants naturally grown in Umudurunna Abba, Nwangele LGA, during the morning hours between 5.30 pm - 6.30pm, in May 2018. The specimens were collected during the evening period, at the time when the leaves were freely falling off the tree. The leaves were packaged in a bag and taken to the Nnamdi Azikiwe University Agulu campus, Anambra state, the next morning for identification at the department of Biological Sciences, of the same University. Afterwards, the *Terminalia catappa* leaves were washed thoroughly in distilled water and a known quantity (850gm) were dried at room temperature for 1 week. The red and green leaves were separately ground into fine powder form using clean laboratory motor and pestle. 850g of the pulverized plant sample was macerated in 2.5L of methanol for 48 hours with intermittent shaking. It was filtered using a muslin cloth and further filtered through cellulose filter paper. The filtrate was concentrated using rotary evaporator (Buchi Labortechnik) under reduced pressure at 40°C.

## 3. Collection of Parasites

Adult *Onchocerca volvulus* nematodes were obtained from the contents of the skin nodules of one hundred volunteers / afflicted individuals from Umuokwara, Ugwuaku and Aku ihube villages in Okigwe LGA, Imo state Nigeria. The volunteer donors aged between 29 – 65 years old, have lived in the villages of Ezinnachi Autonomous Community in Okigwe LGA (Umuokwara, Aku ihube, and Ugwuaku villages) for ten years and above and have not received any medication for Onchocerciasis in the past 5 years and thereby qualified the study criteria.

Every potential donor who showed up for the screening procedure was welcomed. The choosing of study sample was without bias for gender, sex, occupation and stratification. The ten donors provided written formal consent.

Skin snip biopsy and microscopic investigation to confirm presence of microfilariae load were performed for diagnosis. The Skin biopsy test was after a simple palpitation test conducted to ascertain that the nodular content was possibly the *Onchocerca volvulus* nematode. The content of the nodule was pressed with the index finger and observed for a movement. Movement indicated the presence of the *Onchocerca worm*. The *Onchocerca volvulus* was sourced via nodulectomy procedure and for which head nodules were particularly targeted. This study was approved by the Ethics Committee of the Imo state government. (Protocol number: 105/2014).

Skin snip biopsy was first performed on the identified individuals by elevating a small cone of skin (3 mm in diameter) with a needle and shaving it off with a scalpel. This resulted in the removal of around 2 mg of tissue. The tissue was then incubated in normal saline at room temperature for 24 hours to allow the microfilariae (larvae) to emerge. The microfilariae were then identified microscopically. The sites for the skin snip were over the scapula and the lower extremities. A day later, nodulectomy was performed on the patient that enabled the identification of macrofilariae (adult worms) in the tissue.

The extracted bundle of worms still in its natural sac was further preserved in 0.5% carboxymethyl cellulose and taken to the department of pharmacy laboratory of Nnamdi Azikiwe University, Agulu campus, where it was authenticated by a parasitologist. The sac was then incised to recover the nematodes, which were placed in a beaker containing 0.5% carboxymethyl cellulose. A total of 252 worms were collected.

## 4. Weighing of Worms

Using an electronic weighing instrument (OHAUS), the *Onchocerca volvulus* (worms) were weighed individually before introduction into the methanolic extract beaker and individually weighed again upon death. The differences in weights of individual worms were recorded. This was done to assess the possible mechanism of action of the methanolic *Terminalia catappa* leaf extract using the parameter of weight.

## 5. Data Analysis

The time of paralysis and death of the *Onchocerca volvulus* of the various extract treatment are given in a table. Paralysis occurred when the worms do not revive when vigorously shaken. Death was recorded when the worms lost their motility followed with fading away of their body colour. The results are dose-dependent. The collective weight of the worms before and after death was recorded to determine possible discrepancy.

## 6. Result

The methanolic *Terminalia catappa* leaf extracts effect on *onchocerca volvulus* recorded the following mean average results. The dose of 40mg/ml leaf extract showed  $0.005 \pm 0.0001$  weight reduction of worms upon death. The dose of 20mg/ml and 10mg/ml leaf extract recorded  $0.003 \pm 0.0001$  and  $0.002 \pm 0.000$  weight reduction respectively. The reference drug (Albendazole) showed a reduction in the mean average weight of worms ( $0.005 \pm 0.000$ ), upon death

compared to the live weight. This weight reduction was observed as a consistent pattern with the *T. catappa* extract. And worms in Saline water recorded a weight increase of  $.001 \pm 0.006$  upon dead instead.

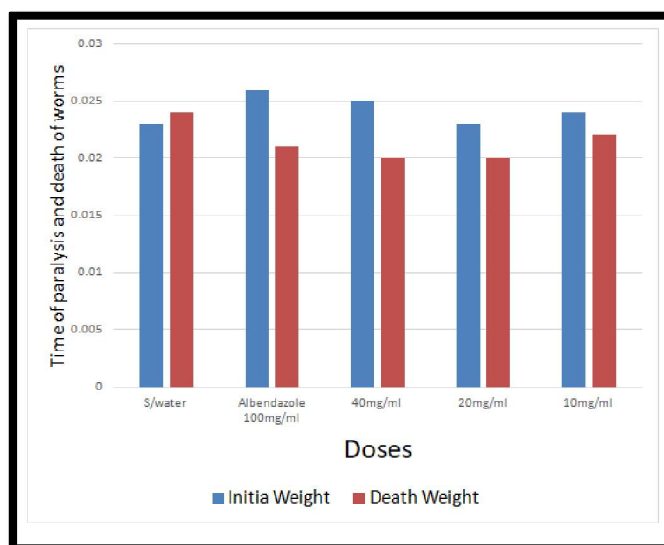


Figure 1: Determination of Osmotic Effect of *Terminalia Catappa* Linn. Leaf Extract on *O. Volvulus* Using Parameter of Weight

## 7. Conclusion

This study showed a consistent pattern of the *Onchocerca* nematodes weight reduction upon recording death in the methanolic *Terminalia catappa* leaf extract. The alteration in weight of nematodes upon death indicates that the *Terminalia catappa* leaf extracts increased osmolarity between the internal and external environment of the worms. The reduction in weight of worms upon death would mean that there was a fluid loss from the body. The result agrees with the study of Nurulaini *et al.* 2011. This could be because of the metabolite constituent of the *Terminalia catappa* leaf extract. Therefore, it can be concluded that the anthelmintic efficacy of the *Terminalia catappa* leaf is more potent than that 100mg Albendazole. More researches are necessary to authenticate this mechanism of osmolarity action reported herein.

## 8. Availability of Data and Materials

The dataset(s) and other materials can be accessed upon request via the authors email handler (please put your email)

## 9. Ethics Approval and Consent to Participate

An ethical clearance and approval was obtained from the the Ethics Committee, public health department Imo State Ministry of Health Owerri (Protocol number: 105/2014).

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