

# Effect Of Fractions of Methanolic *Terminalia Catappa* Linn. (Green Leaf) Extract on *Onchocerca Volvulus* by Modified Selected in -Vitro Bioassay

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**Abstract- Purpose:** Adult *Onchocerca volvulus*, the causative agent of onchocerciasis were introduced into fractions of Methanolic *Terminalia catappa* Linn (Tropical Almond) green leaf extract and observed for paralysis and death.

**Method:** A total of 252 *Onchocerca* worms sourced via nodulectomy were used for the study from April 2018 to February 2019. Green leaves of *Terminalia catappa* were crushed and the phytochemical constituents extracted using 95% ethanol, then filtered and evaporated (Tabassam method). The dried extracts were dissolved in sterile dilution and prepared in *T. catappa* fractions of Methyl acetate, Butanol and N-hexane doses of 20mg/ml and 40mg/ml concentration. Six labeled beakers were used for the study and six worms assigned per beaker. A positive control beaker contained Albendazole (anthelmint) and a negative control beaker, contained saline water. The worms were observed for paralysis and death time as the procedure was repeated three times.

**Result:** Ethyl acetate (20mg/ml) paralyzed the worms at  $73 \pm 10.27$  mins and killed at  $87 \pm 4.72$  mins. The 40mg/ml dose of ethyl acetate paralyzed the worms at  $61 \pm 1.73$  mins and death at  $75 \pm 2.08$  mins mean average time. Butanol fraction shows that 20mg/ml dose paralyzed worms at  $92 \pm 1.52$  mins and killed the worms at  $111.3 \pm 4.17$  mins while the 40mg/ml dose paralyzed the worms at  $81 \pm 3.46$  mins and recorded the death at  $102 \pm 4.80$  mins mean average time. N-hexane fraction of 20mg/ml dose paralyzed the onchocerca worms at  $107 \pm 8.8$  mins and recorded the death at  $131 \pm 4.05$  mins. And the 40mg/ml dose of n-hexane paralyzed the worms at  $98 \pm 3.21$  mins and killed at  $112 \pm 9.02$  mins. All results are statistically significant at  $P > 0.05$ . Positive control (Albendazole) showed mean average *O. volvulus* paralysis time of 58 mins  $\pm 8.96$  mins and death at  $93.7 \pm 9.59$  mins. The average negative control (saline water) showed a

mean average paralysis time of *O. volvulus* at  $154 \pm 5.97$  and death at  $235 \pm 4.21$  mins.

**Conclusion:** Conclusion is that ethyl acetate fraction is of concentration dependent anthelmintic effects on *Onchocerca volvulus*

**Indexed Terms-** *Terminalia catappa*, *Onchocerca volvulus*, Methyl acetate, Butanol, N-hexane,

## I. INTRODUCTION

Onchocerciasis is recognized as a major deleterious disease of massive public health and socioeconomic concern<sup>[21]</sup>. It is classified as a Neglected Tropical Disease by the World Health Organisation (WHO), with hundreds of thousands of people blind and an increasing number of individuals at risk of more infection<sup>1</sup>. Empirical findings revealed that it is the second leading cause of blindness caused by infection with the *Onchocerca volvulus* nematode<sup>[6]</sup>. This disease has ravaged the Simulium damnosum (black fly) endemic local communities of South Eastern Nigeria<sup>[1]</sup> About 99% of cases are found in Africa where 85million people live in endemic areas<sup>[2]</sup>. Nigeria has the highest number of persons with onchocerciasis, accounting for over one-third of the global prevalence<sup>[3]</sup>. 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<sup>[4]</sup>.

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 by the [2] that 4 billion people (80% of the world population) presently use herbal medicine for health-care purposes. Most of these plants contain potent active compounds that inhibit and cure several ailments [7].

*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].

## II. MATERIALS AND METHODS

The study is an experimental study involving two phases, which are pre-survey logistics and parasitological / *Terminalia catappa* investigations. A sample of the *Terminalia catappa* leaf gotten from the traditional medicine practitioners was taken to the pharmaceutical and toxicology laboratory of the Department of Pharmacy, Nnamdi Azikiwe University Akwa, Agulu campus, Anambra state Nigeria, for acute toxicity test and phytochemical analyses. The *In-vitro* experimentation (conducted in laboratory beaker) with the adult nematode *Onchocerca volvulus* as to the activity of *Terminalia catappa* extracts was conducted with the supervision of the laboratory scientist of the pharmaceutical and toxicology department of the Nnamdi Azikiwe University Agulu, Anambra state Nigeria.

### Purchase Albendazole / Reagents

The drug Albendazole 100mg (anthelmintic) was purchased from a registered pharmaceutical store, Hashem Pharmacy in Owerri, Imo state. While the other reagents used for the study as n-hexane, butanol, methanol, distilled water, etc., were purchased from a local chemical store dealer. The laboratory mice were sourced from the Zoology department of Nnamdi Azikiwe University, Agulu campus, Anambra state.

### Collection and Extraction of Leaf

*Terminalia catappa* (Combretaceae) 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 identified at the department of Biological Sciences of Nnamdi Azikiwe University, Agulu campus, Anambra state. Afterwards, the *Terminalia catappa* leaves were washed thoroughly in distilled water and a known quantity (850gm) were dried at room temperature for 1 week.

### Preparation of Fractions

Methanol extract was prepared following the methods of Tabassam [16]. The samples were sequentially subjected to liquid-liquid partition method [17], with n-hexane, followed by ethyl acetate, then n-butanol, resulting in three fractions with different polarities: the hexane fraction (FHEX), which was the least polar fraction; the ethyl acetate fraction (FAcOEt) the most polar fraction; and the n-butanol fraction (FBuOH), with intermediate polarity. 100mg/ml of the crude extract in methanol was mixed with 150ml of distilled water and was poured in a separate funnel. 500ml of n-hexane was poured into the funnel and shook vigorously, releasing pressure at intervals. It was allowed to stand for 30 minutes for proper separation. Then the fraction was collected in a clean beaker. 500ml of ethyl acetate was poured in the residue and was shaken vigorously. It was also allowed to stand proper separation upon collection in a clean beaker. Then the butanol was finally poured in the residue, shook and then allowed to stand for 30 minutes for separation and collection. Finally, the resulting fractions were collected using a water bath at 40°C for further use.

### 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 Akuihube 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, Akuihube, 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.

Skin snip biopsy and microscopic investigation to confirm presence of microfilariae load were performed for diagnosis. The Skin biopsy test was after a simple palpation 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.

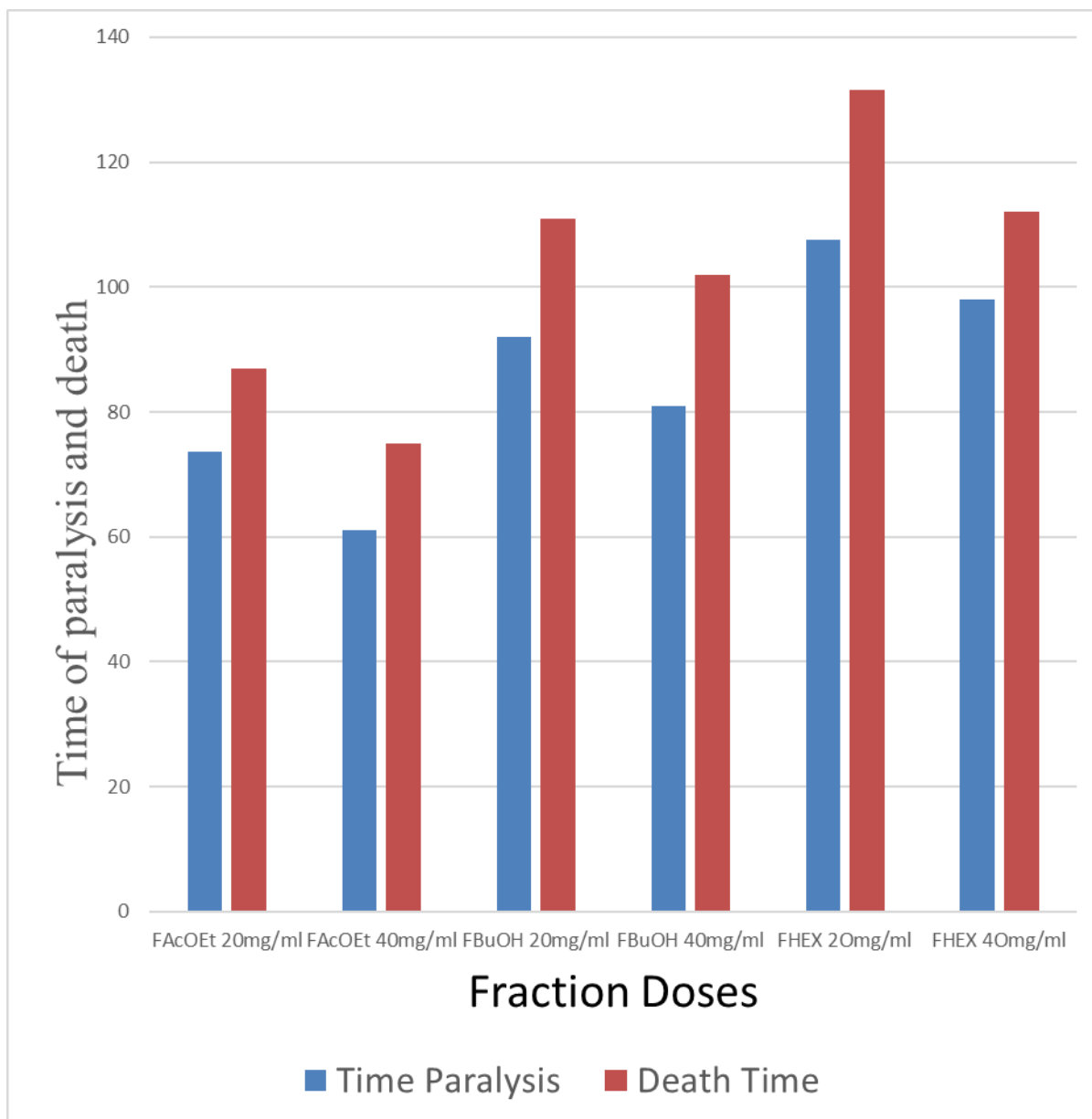
### Data Analysis

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, on comparing the fractions of methanolic extracts of the plant leaf showing the highest anthelmintic activity with death time. After treatment observation with fractions of ethyl acetate, n-hexane, and butanol was also tested for the polar constituents,

to determine the more potent fraction. Each experiment was performed in triplicate and results were expressed as Mean  $\pm$  Standard Deviation (SD). To evaluate the effects of the various fractions on *Onchocerca volvulus*, the Shapiro-Wilk test for normality was applied. Once the results were confirmed to be normally distributed, ANOVA followed by Dunnett's test analyzed data obtained. The significance level was set at 5% for all tests

### Results

The effect of methyl acetate, butanol, and N – hexane fractions of methanolic *T. catappa* (green leaf) extract on *O.volvulus* revealed to be dose concentration-dependent. Methyl acetate, butanol, and N – hexane fractions of methanolic *T.catappa* leaf extract also showed maximum efficacy at a concentration of 40mg/ml for this study, which is comparable to the reference drug (Albendazole 100mg). Observation with the fraction doses showed that ethyl acetate is more potent on *O. volvulus* at mean average paralysis time of 122.5mins and death at 181.5mins. Positive control (Albendazole) showed mean average *O. volvulus* paralysis time of 58 mins  $\pm$ 8.96mins and death at 93.7 $\pm$  9.59mins. The average negative control (saline water) showed a mean average paralysis time of *O. volvulus* at 154 $\pm$ 5.97 and death at 235 $\pm$ 4.21mins. Results further showed that methyl acetate fractions of methanolic *Terminalia catappa* red leaf extracts are less potent than the standard drug Albendazole. The research work recorded 100% mortality in different concentrations, of the total 36 *Onchocerca volvulus* (nematodes) used for the research work. All results are statistically significant at  $P>0.05$



Bar chart 1: Effects of fractions of methanolic *T.catappa* green leaf extract on *Onchocerca volvulus* using parameters of paralysis and death

Key indications

FAc OEt – Fraction of Ethyl acetate

FBuOH – Fraction of Butanol

FHex – Fraction of Hexane

### CONCLUSION

Observation with the fraction doses showed that ethyl acetate is more potent on *O. volvulus* than the other methanolic fractions of green leaf extracts, at mean average paralysis time of 122.5mins and death at 181.5mins. Positive control (Albendazole) showed mean average *O. volvulus* paralysis time of 58 mins

$\pm 8.96$ mins and death at  $93.7 \pm 9.59$ mins. Results further showed that ethyl acetate fractions of methanolic *Terminalia catappa* green leaf extracts are less potent than the standard drug Albendazole.

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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)

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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