

Anthelmintic Effects of Terminalia Catappa (Tropical Almond) Leaf Extract on Onchocerca Volvulus By Modified Selected In-Vitro Bioassay

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ABSTRACT

Background: the extract of Terminalia catappa Linn. (Tropical Almond) has been investigated in various pharmaceutical studies and is known to contain a variety of chemical components. The plant extracts exhibit anthelmintic as well as biological activities, including antioxidants.

Purpose: The study was to determine the effects of various concentrations of Terminalia catappa (green and red) leaf extract on the adult Onchocerca volvulus, by modified selected in-vitro bioassay, using the parameters of paralysis and death.

Method: A total of 252 adult worms (Onchocerca volvulus) sourced via nodulectomy were used for the study. Diagnosis was done by palpating the patient's skin nodules, feeling for hardness and movement of the nodule content. The crushed red and green leaves of Terminalia catappa was extracted using 95% ethanol, filtered, and evaporated (Tabassam method). The dried extracts were also dissolved in sterile dilutions and prepared in a final concentration doses of 10mg/ml, 20mg/ml and 40mg/ml. A positive control beaker contained Albendazole and a negative control beaker, contained saline water.

Result: Terminalia catappa red and green (combined) leaf extract recorded a mean average paralysis time of *O. volvulus* at 15.5mins and death at 39.2mins. 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.

Conclusion: is that methanolic extract of Terminalia catappa leaf possesses concentration dependent anthelmintic effects on Onchocerca volvulus and is more potent than the reference drug (Albendazole)

Keywords: Terminalia catappa, Onchocerca volvulus, Albendazole, anthelmintic

I. 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 Organisation (WHO), with hundreds of thousands of people blind and an increasing number of individuals at risk of more infection. 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 with no cure yet. This disease 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. According to^[7], 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. 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]. There have been studies done previously that suggested that the most polar fractions gotten from *T. catappa* leaves are effective against bacteria^[13] and fungi^[14].

II. MATERIALS AND METHODS

Study Area Design

The study area is Ezinachi Autonomous Community in Okigwe Local Government Area,

which is one of the present 27 Local Governments Areas in Imo State, (Nigeria) created in 1976.

The study is an experimental study involving two phases, which are pre-survey logistics and parasitological / *Terminalia catappa* investigations. The researcher went with the officer in charge of the local government health centre at Okigwe LGA. Both visited the endemic onchocerciasis communities of Umuokwara, Ihubeaku, Ugwuaku villages in Okigwe LGA and oral questions were put to the locals and persons afflicted with the *Onchocerca volvulus* parasite as to the nature of treatment available to them, with particular emphasis on herbal medicines.

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/ Lab Mice

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. 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^oc 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.

Phytochemical Screening

All extract of the plants were analyzed for the qualitative and quantitative phytochemicals analysis using standard methods. The phytochemical analysis include; test for Tannins, test for Saponin, test for flavonoids, test for steroids, test for Cardiac glycosides (Keller-killing test), Alkaloid determination, and Saponin determination.

Ethical Consideration

An ethical clearance was obtained and approved from the Ethics Committee, public

health department Imo State Ministry of Health Owerri (Protocol number: 105/2014).

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 methanolic extracts of the plant leaf showing the highest anthelmintic activity with death time

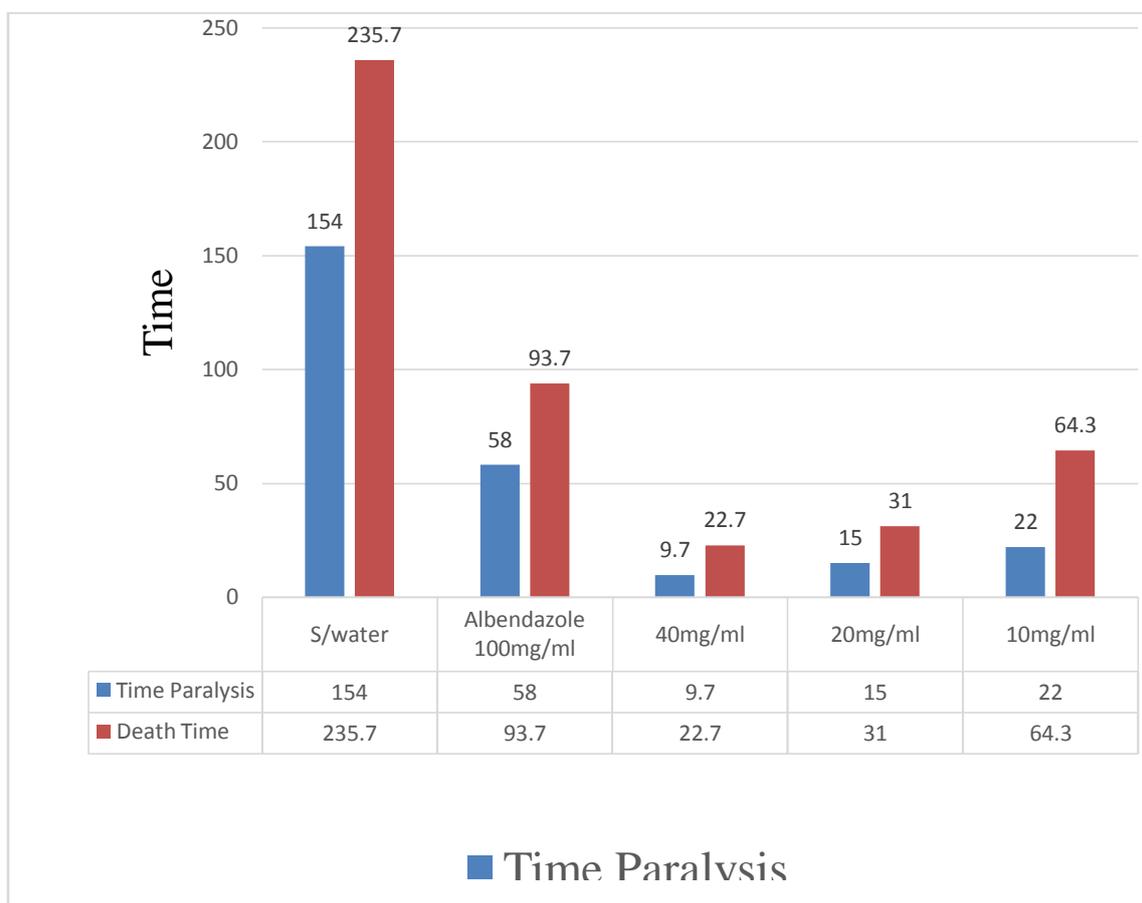
The collective weight of the worms before and after death was recorded to determine possible discrepancy. After treatment observation with fractions of ethyl acetate, n-hexane, and butanol was also tested for the polar constituents, to determine the more potent fraction.

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 Dunnet's test analyzed data obtained. The significance level was set at 5% for all tests

III. RESULTS

The anthelmintic activity of the *T. catappa* (the red and green combined) leaf extract, on *O.volvulus* revealed to be dose concentration-dependent. The anthelmintic activity of *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). Results further show that the combined methanolic *Terminalia catappa* leaf extracts are more potent than the standard drug Albendazole and that more weight is lost with the leaf extracts and the potency of the methanolic leaf extract was more efficient than the reference drug Albendazole. The research work recorded 100% mortality in different concentrations, of the total 252 *Onchocerca volvulus* (nematodes) used for the research work.

Fig. 1(Bar chart) shows the effect of methanolic *Terminalia catappa* leaf extract on *O. volvulus* using the parameters of paralysis and death. Standard drug Albendazole is a Positive Control and Normal saline was used for Negative Control. The Positive Control for *O. volvulus* paralysis is 58 \pm 8.96mins and death at 93.7 \pm 9.59mins. The Negative Control for *O. volvulus* paralysis was recorded at 154 \pm 6 and death at 93.7 \pm 9.6mins.



Bar Chart 1: Efficacy of T. catappa red and green (combined) leaf on O. volvulus using parameters of paralysis and death.

The red and green Terminalia catappa leaf (combined) extracts recorded paralysis of O.volvulus at a mean average time of 15.5mins and death time at mean average 31mins.

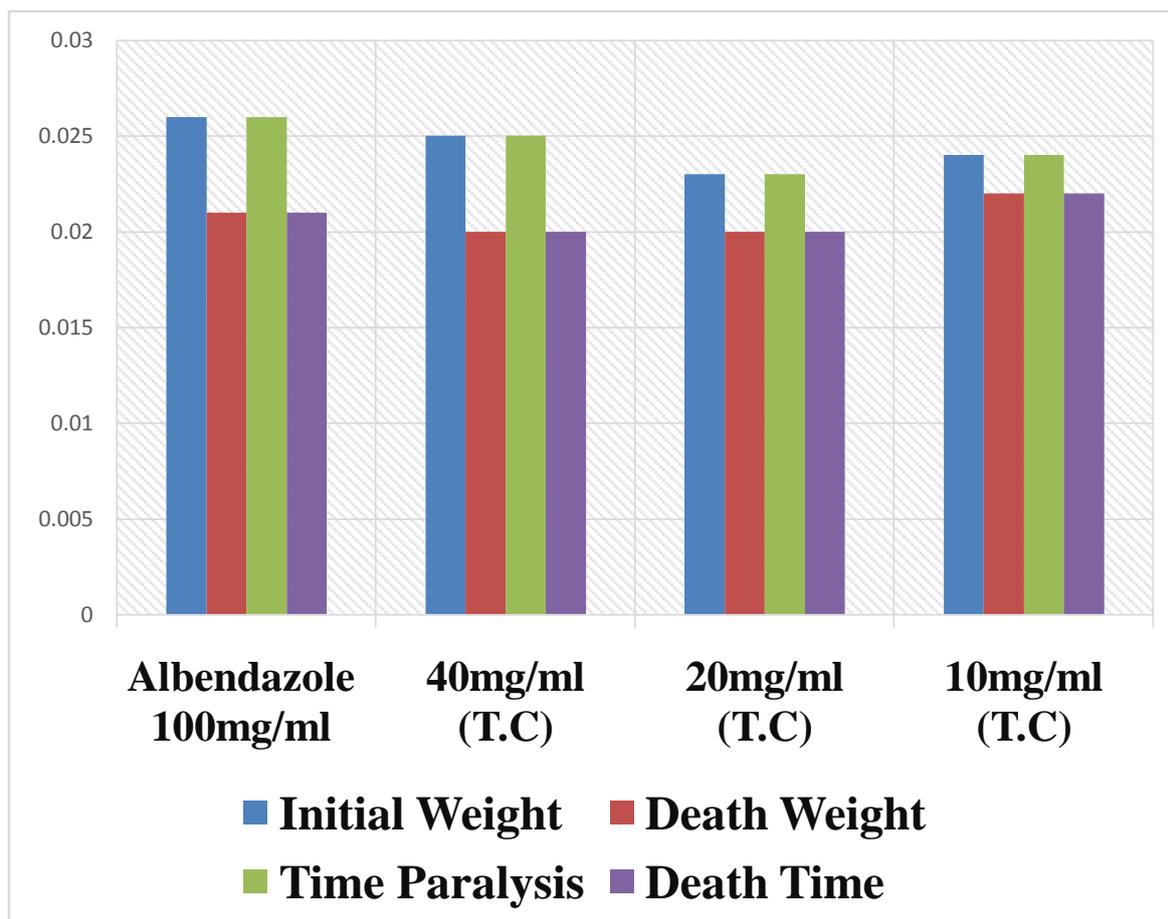
The dose concentration of 40mg/ml of T. catappa extract recorded the fastest time for O. volvulus paralysis at a mean average of 9.7±1.20mins and death at 22.7±1.45mins. The doses concentration of 20mg/ml leaf extract recorded O. volvulusparalysis time of 15.0±1.53mins and death at 31.0±1.56mins.

And the leaf extracts concentration dose of 10mg/ml, recorded paralysis at 22.0±7.52mins and death at 64.3±5.93.

The result shows the efficacy of Terminalia catappa combined red and green leaf (combined) extract to be dose-dependent.

All results are statistically significant at P>0.05
Comparing the effect of Albendazole and Terminalia catappa leaf extract on Onchocera volvulus, using the parameters of paralysis /death and weight.

Fig. 2 (Bar chart) shows comparison between the anthelmintic activity of (reference drug) Albendazole and metanolic Terminalia catappa leaf extract, using the parameters of paralysis /death time and weight.



Bar chart 2. Comparing the effect of Albendazole and Terminalia catappa leaf extract on Onchocerca volvulus, using the parameters of paralysis /death and weight.

The paralysis time of nematodes in the anthelmintic drug Albendazole is 58 ± 8.96 and the death time at 93.7 ± 9.59 minutes. While the mean average paralysis time of Onchocerca volvulus with methanolic Terminalia catappa leaf extract is 15.5 minutes and death, at a mean average time of 39.3 minutes.

Fig 2 also shows results in respect to the effects of Albendazole and Terminalia catappa leaf extract on *O. volvulus* using the parameter of weight. *T. catappa* shows a reduction effect on *O. volvulus* in initial mean average weight of 0.024 gm to the final 0.020 gm upon death. While the reference drug (Albendazole) recorded a reduction in weight of *O. volvulus* from the initial 0.026 to the final 0.021. The difference in weight loss is slightly higher with the methanolic Terminalia catappa leaf extract, though the result may not be statistically significant.

IV. DISCUSSION

The methanolic extract of Terminalia catappa leaf was subjected to Phytochemical

screening to identify the classes of natural products which may be responsible for the effect on Onchocerca volvulus. The leaf of Terminalia catappa contains phytochemicals such as steroids, polyphenols, flavonoids, triterpenoids, saponins, glycosides and tannins and the results of the research is the same as the report of [18].

This investigation revealed the presence of resins, phenols and triterpenes steroid in ethyl acetate, n-hexane, and butanol. Tannin and saponins were seen in ethyl acetate and butanol. Flavonols were seen in n-hexane and butanol. This result is the same with the study on Phytochemical Activity of Terminalia catappa leaves by [19]. A similar study by [14] showed the presence of the same phytochemical constituents of crude Terminalia catappa leaf extract.

The Fig 2 result shows the anthelmintic activity of the *T. catappa* (the red and green combined) leaf extract, on *O. volvulus* which results showed is dose concentration-dependent. There was a corresponding decrease in anthelmintic

activity of Terminalia catappa leaf extract as the doses concentration decreased. The anthelmintic activity of T.catappa leaf extract showed maximum efficacy at a concentration of 40mg/ml for this study, which is comparable to the reference drug (Albendazole 100mg).

The combined potency of T. catappa red and green leaf extracts recorded paralysis at a mean average time of 15.5mins and death at a mean average time of 31mins. The combination showed 100% mortality of Onchocerca volvulus at doses concentration of 40mg/ml, 20mg/ml and 10mg/ml. This result collaborate a lot on other similar studies, such as that by [20], who reported that the anthelmintic activity of the T. catappa leaf was due to the presence of alkaloids. [21], have also reported the anthelmintic activity of condensed tannins (proanthocyanidins). Besides tannins, phenolics and flavonoids, other plant secondary compounds such as saponins, and terpenoids steroids may also have contributed to the anthelmintic activity in vitro [22].

Fig. 2 shows that the combined methanolic Terminalia catappa leaf extracts are more potent than the standard drug Albendazole. The result agrees with the study of [23]. More weight is lost with the leaf extracts and the potency of the methanolic leaf extract was more efficient than the reference drug Albendazole. Therefore it can be stated that methanolic Terminalia catappa leaf extracts, showed a more significant anthelmintic activity compared to the reference drug Albendazole. This could be because of the metabolite constituent of the Terminalia catappa leaf extracts.

V. CONCLUSION

From the research work, it can be concluded that crude methanolic extract of Terminalia catappa leaf, possess concentration dependent anthelmintic effects on Onchocerca volvulus. It can become a potent key ingredient of anthelmintic herbal drug formulation and a safe, sustainable, environmentally friendly and affordable treatment option for Onchocerca volvulus infection.

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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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