

Therapeutic Efficiency of Selected Ethnomedicinal Plants and Medications for *Onchocerca volvulus* Infection Endemic in Imo State, Southeastern Nigeria

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ABSTRACT

Onchocerca volvulus infection – Onchocerciasis – is a priority tropical disease that has been targeted for elimination by World Health Organization (WHO). The Standard strategy to combat this chronic parasitic infection is the administration of Annual Community Directed Treatment with Ivermectin (CDTi), yet high prevalence rates and transmission persist.

Alternative strategies are therefore needed to achieve Onchocerciasis elimination where CDTi effectiveness is suboptimal. Hence the need for this research work on elimination and control of Onchocerciasis. This study was a cross-sectional experimental study carried out to investigate the therapeutic efficiency of some selected treatment options of Onchocerciasis prevalent in selected endemic areas of Imo State, Southeastern Nigeria. The multistage sampling technique was adopted to select samples for the study. All infected subjects used for this study gave an informed consent to be part of the study. Accurate screening methods were used to identify subjects with detectable microfilariae that were positive and highly sensitive to the diagnostic tests. A total of one thousand five hundred and thirty (1,530) inhabitants of the studied communities (Umulolo, Amuro, Ihube,

Okwe, Umuna, Ezelu, Nzerem, Umuihi, Umuneke and Umulewe) were screened for the infection. Out of these, the number infected by *Onchocerca volvulus* in the ten studied areas were five hundred and ninety-two (592). The infected subjects were sampled evenly and assigned into subgroups to receive treatment with doses of Ivermectin, Doxycycline as well as *Morinda lucida* and *Indigo tinctoria* plant extracts. Samples from the treated subjects were collected, screened and assessed for elimination of microfilarial loads. Results showed that higher number of infected subjects (91.1%) benefited from treatment with oral dose of Doxycycline and had their clinical signs disappeared as compared with 82.96% (Ivermectin), 47.2% (*Morinda lucida*) and 46.5% (*Indigo tinctoria*) benefited subjects. Statistical analysis using the Duncan Multiple Range Test also showed significant differences $p \geq 0.01$ in the treatment options administered. It was however recommended that more improved treatment options are needed to ensure massive control measures and total eradication of the infection.

Keywords: Onchocerciasis, therapeutic, microfilariae, screening, endemic, elimination.

INTRODUCTION

Onchocerciasis is a neglected tropical disease and the second leading cause of infectious blindness worldwide; approximately 500,000 people are blind due to this disease. Its epidemiologic patterns of infection differ between Savannah and forest regions [1,2]. The disease affects rural communities in Tropical and Sub-tropical regions and is a major cause of blindness and skin disease in endemic areas with serious socio-economic consequences [3,4]. Human onchocerciasis is a serious neglected tropical disease caused by the filarial nematode *Onchocerca volvulus* that can lead to blindness and chronic disability. Control of the disease relies largely on mass administration of a single drug- the Annual Community Directed Treatment with Ivermectin, and the development of new drugs and vaccines depends on a better knowledge of the parasite biology. The filaria are a group of tissue-dwelling parasitic nematodes of vertebrates that are spread by blood-feeding arthropods (*Simulium damnosum*)

[5,6]. *Onchocerca volvulus* is the most pathogenic agent of onchocerciasis (or ‘River

Blindness’), a leading cause of morbidity and socioeconomic loss for the world's poorest populations. Approximately 17 million people are still infected with *O. volvulus*, predominantly in Africa. Infections are chronic and manifest clinically as debilitating skin disease and in 1.2 million people vision impairment or blindness is paramount. First-stage larvae, known as microfilariae (L1/mf), are produced by fertile female worms residing within onchocercomata (nodules). They migrate to the skin and other organs (for example, the anterior chamber of the eye), where they induce inflammatory reactions that are responsible for most *Onchocerca*-related pathology [7]. Onchocerciasis was identified by the World Health Organization (WHO) as

a potential candidate for disease elimination through annual (or semiannual) mass drug administration (MDA) of ivermectin. The standard treatment for onchocerciasis is ivermectin (150- μ , g/kg given orally ever 6 to 12 months). Ivermectin is a highly lipophilic, 16-membered macrocyclic lactone from *Streptomyces avermitilis*. Single dose ivermectin effectively kills microfilariae by blocking postsynaptic, glutamate-gated chloride ion channels, inhibiting transmission, and paralyzing the nematode. It also appears to enhance immune responses against *O. volvulus* in the treated host. Other than a significant oncogenic effect on adult female worms, ivermectin has little macrofilaricidal effect; therefore, it controls but does not cure the disease. Ivermectin is solely microfilaricidal, which means it must be given over decades, past the lifespan of the long-lived adult worms. Moreover, in much of Central Africa where *Loa loa* is co-endemic with *O. volvulus*, ivermectin cannot be used due to the risk of *Loa*-associated irreversible neurological severe adverse events and death. Despite its success in Latin America and small foci in Africa, elimination of onchocerciasis in Africa is unlikely to be achieved within the proposed timeframes solely through MDA with ivermectin. Reliance on a single drug also increases the potential for the emergence of ivermectin-resistant *O. volvulus*, making the development of new drugs or novel therapies imperative [6,7].

Plants have been reported as alternatives by most researchers because they have long history of extensive use in traditional medicine for the management of various diseases. The traditional claims on the healing properties of plants are most often tied to cultural reasons, long-term use, affordability, safety and efficacy. Ethnomedicinal plants have also played significant role in drug discovery, development and production [8]. Two of such

important and widely used ethnomedicinal plants in sub-Saharan Africa are *Morinda lucida* Benth which belongs to the Rubiaceae family and *Indigofera tinctoria* Linn which belong to the family Leguminosae. Recently, the Rubiaceae family has been described to be made up of 576 genera and 12,000 species and based on phylogenetic approach to plant systematics, this family appear to represent the fourth largest angiosperm family after Asteraceae (23,000 species), Orchidaceae (19,500 species) and Fabaceae. (18,000 species).^[9] Based on molecular phylogenetic studies, the Rubiaceae family has been subdivided into three subfamilies namely Cinchonoideae, Ixoroideae and Rubioideae.^[10] In sub-Saharan Africa, there are 1900 species of the Rubiaceae family belonging to 164 genera, with over half of them documented to be native to the continent.^[11] *M. lucida* belongs to the Rubioideae subfamily, Morindeae and genus *Morinda* while *I.tinctoria* belong to the Leguminosae subfamily, Fabaceae. Natural products derived from plants have served as the primary healthcare needs of millions of indigenous people for centuries, many of which have been documented and scientifically validated.

MATERIALS AND METHODS

The study was a cross sectional experimental study carried out in ten rural areas of Imo State, Nigeria where *Onchocerca volvulus* infection is endemic. The multistage sampling technique was adopted to select samples for the study. All subjects used for this study gave an informed consent to be part of the study. Data was uploaded into the SPSS version 21 software and the Duncan Multiple Range Test was used to compare and analyse the therapeutic efficiency of the treatments and the research null hypothesis tested at the level of significance ($p > 0.01$).

Preparation of *Mucida lucida* and *Indigofera tinctoria* extracts using Decoction & Maceration Methods.^[12]

- i. Before being processed, the fresh plant leaves were properly hand cleaned by separating foreign materials that are not suitable for extract.
- ii. The leaves were then dried under the sun for few days and grinded into powdered forms which will be used as dried herb samples.
- iii. Large quantity of dried herbs was placed into two separate small sauce pan and covered with large amount of cold water to submerge the dried herbs and the mixtures stirred consistently.
- iv. The mixtures were slowly heated and brought gently to boiling point.
- v. A lid was placed over the two separate pots to allow for simmering for 20-45 minutes.
- vi. The heat was removed and the mixtures allowed cooling to room temperature.
- vii. The mixtures were then carefully strained off to separate the extracts from the mixture into different clean jars ready for maceration.
- viii. The filtrates were then separately concentrated under reduced pressure using a rotary evaporator at 55C to yield a crude semisolid mass, which were stored differently in a refrigerator at 4C until required.

Procedure for the Administration of Ivermectin (Ivm) and Doxycycline (Dox).^[13]

Eligible subjects with *Onchocerca volvulus* infection from the initial sample collection from the ten selected communities served as a control group. Subjects were further

randomly assigned into sub-groups to receive treatments for Onchocercal infection. The first sub-group were assigned to receive a single dose of Ivermectin for duration of 6 months. Samples of Skin Snip and blood were collected and screened to check for the elimination of pathogen load (*Microfilaria* and adult worm) load and confirmatory results recorded.

Subjects assigned to the second sub-group were given treatments with Doxycycline orally at a dose of 200mg daily for 4 – 6 weeks. Samples from doxycycline - treated subjects were collected, screened and assessed after 6 weeks to check for elimination of microfilarial levels and adult worms. Confirmatory results were also recorded and analysed.

Procedure for Administration of *Morinda lucida* and *Indigofera tinctoria* Extracts.^[8]

Another third and fourth group were subjected to the administration of *Morinda lucida* and *Indigofera tinctoria* extracts. One hundred gramme (100g) of the macerated samples were dissolved in 1000ml of distilled water to make 100g/l per subject. Each subject was given one glass cup daily for 6-8weeks. Samples from the treated subjects were collected, screened and assessed for elimination of microfilarial load. Results were recorded and also analyzed.

RESULTS

A total of one thousand five hundred and thirty (1530) inhabitants of the studied communities (Umulolo, Amuro, Ihube, Okwe, Umuna, Ezelu, Nzerem, Umuihi, Umuneke and Umulewe) were screened for the infection. Out of these, the number infected by *Onchocerciasis* *volvulus* in the ten studied areas were five hundred and ninety-two (592). The infected subjects were sampled and evenly assigned into subgroups to receive treatment with doses of Ivermectin, Doxycycline as well as *Morinda lucida* and *Indigo tinctoria* plant extracts. Samples from the treated subjects were

collected, screened and assessed for elimination of microfilarial loads. Results showed that higher number of infected subjects (91.1%) benefited from treatment with oral dose of Doxycycline and had their clinical signs disappeared as compared with 82.96% (Ivermectin), 47.2% (*Morinda lucida*) and 46.5% (*Indigo tinctoria*) benefited subjects.

Figure 1 showed the disappearance of clinical signs with ivermectin. Out of the 123 (33.3%) number of subjects administered ivermectin, 102 (82.9%) of the participants in all the studied communities benefitted with disappearance of clinical signs and symptoms. This study revealed that subjects administered with ivermectin in Umuihi, Umuneke and Umulewe communities had the highest rates (87.5%) of resolution of clinical signs after ivermectin in take while those in Okwe community had the least rates (70%). Figure 2 showed the disappearance of clinical signs with Doxycycline. Out of the 123 (33.3%) number of subjects administered with Doxycycline, 112 (91.1%) of the participants in all the studied communities had resolution of their clinical signs. Subjects in Umuna and Nzerem communities had the highest rates (100%) of disappearance of their clinical signs and symptoms while subjects in same Okwe community had the lowest rates (80%) after the intake of Doxycycline. Figure 3 showed the disappearance of clinical signs with *M. lucida* extract. Out of the 123 (33.3%) number of subjects, 58 (47.2%) participants benefitted from the consumption of extracts of *M. lucida* in all the ten (10) communities. Subjects in Umulewe community had the highest rates (75%) of resolution of clinical signs after consumption of the extract while subject in Amuro community had the lowest rates (30%). Figure 4 showed the disappearance of clinical signs with *I. tinctoria* extract. Out of the 123 (33.3%) number of subjects, 56 (47.2%) participants benefitted from the consumption of extracts of *I. tinctoria in vivo* in all the ten (10) communities. Subjects in Umulewe

community had the highest rates (74.5%) of resolution of clinical signs after consumption of the extract while subject in Amuro community had the lowest rates (28.5%). Table 1 showed the comparative efficacy of the selected treatments for *Onchocerca volvulus* infection in the studied areas. Doxycycline demonstrated a higher percentage efficacy than ivermectin and the plant extracts when administered to subjects

infected in the studied areas. This showed a complete disappearance of clinical manifestations seen in majority of the infected subjects.

M. lucida and *I. tinctoria* showed a lower percentage efficacy in the infected subjects residing in the selected studied areas with minimal disappearance of their clinical manifestations.

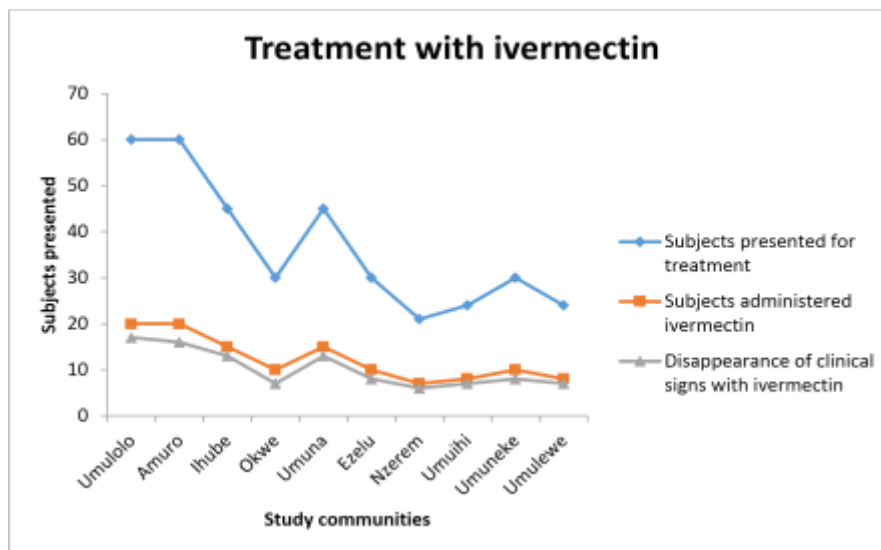


Figure 1: Treatment and disappearance of clinical signs with ivermectin

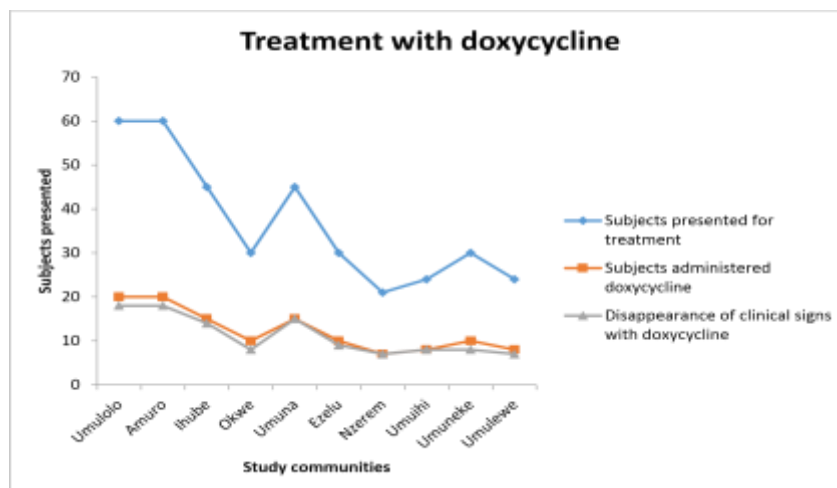


Figure 2: Treatment and disappearance of clinical signs with doxycycline

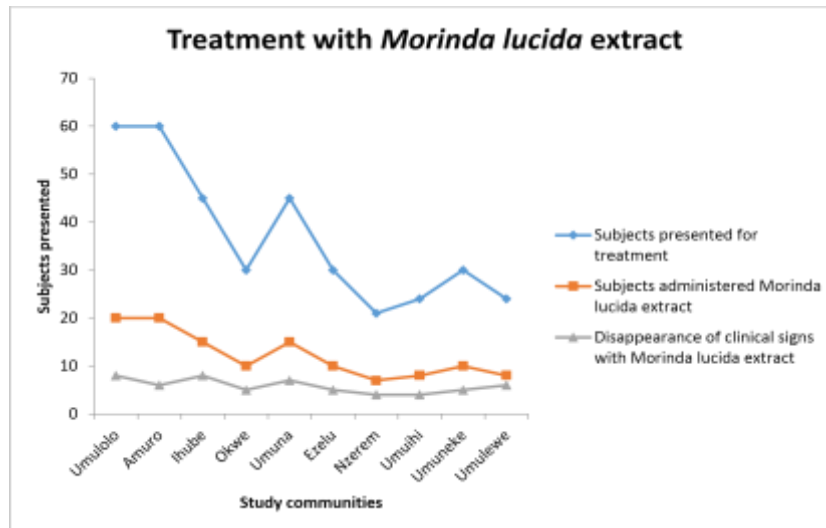


Figure 3: Treatment and disappearance of clinical signs with *Morinda lucida* extract

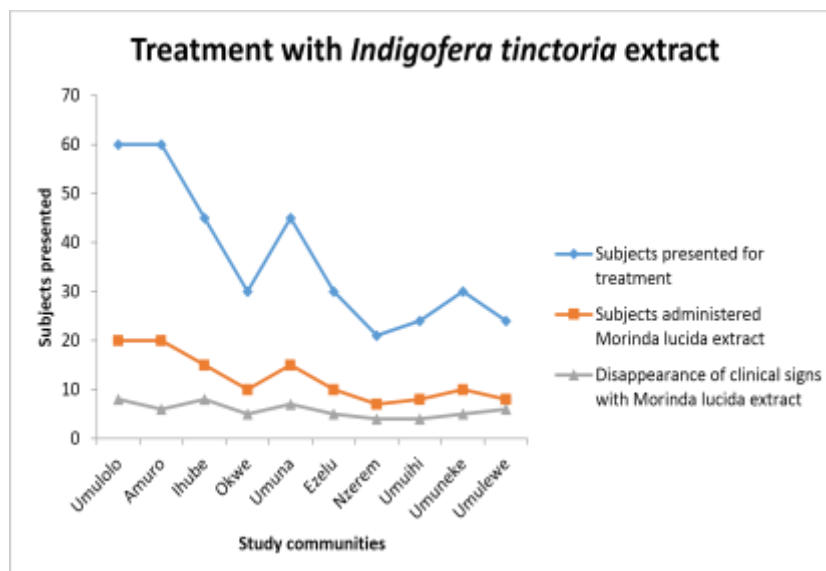


Figure 4: Treatment and disappearance of clinical signs with *Indigofera tinctoria* extract

Table 1: Comparative Efficacy of Selected Treatments for Elimination of *Onchocerca volvulus* Infection in the Studied Areas.

Treatments	Umulolo	Amuro	Ihube	Okwe	Umuna	Ezulu	Nzerem	Umuihi	Umuneke	Umulewe
Ivermectin	85.0 ^b ±9.6	80.0 ^b ±8.8	86.7 ^b ±8.2	70.0 ^b ±7.9	86.7 ^b ±9.1	80.0 ^b ±8.9	85.7 ^b ±9.3	87.5 ^b ±8.4	87.5 ^b ±8.8	87.5 ^b ±9.1
Doxycycline	90.0 ^a ±12.2	90.0 ^a ±13.4	93.3 ^a ±12.8	80.0 ^a ±9.8	100.0 ^a ±15.5	90.0 ^a ±13.6	100.0 ^a ±15.2	100.0 ^a ±15.1	95.0 ^a ±14.1	96.5 ^a ±14.4
M. lucida extract	40.0 ^c ±4.9	30.0 ^c ±4.1	53.0 ^c ±5.4	50.0 ^c ±4.8	46.7 ^c ±4.4	50.0 ^c ±4.9	57.1 ^c ±5.8	50.0 ^c ±4.7	50.0 ^c ±4.5	75.0 ^c ±6.9
I. tinctoria extract	39.5 ^d ±3.7	38.0 ^d ±3.1	50.0 ^c ±5.2	45.0 ^d ±3.7	40.0 ^d ±3.3	50.0 ^c ±4.9	55.4 ^d ±4.9	47.5 ^c ±3.9	48.5 ^c ±4.1	74.5 ^c ±6.2

Mean along the column having different superscript of alphabets differ significantly at $P \geq 0.01$ according to Duncan Multiple Range Test.

DISCUSSION

Onchocerciasis remains a major public health problem in developing countries especially, the Sub-Saharan African countries. Poor health facilities, health infrastructure, and health coverage are the major front-runner factors that made transmission of the disease on-going and protracted elimination. The study investigated the therapeutic efficiency of selected diagnostic treatment strategies in one thousand, five hundred and thirty (1,530) inhabitants of the studied communities (Umulolo, Amuro, Ihube, Okwe, Umuna, Ezelu, Nzerem, Umuihi, Umuneke and Umulewe). Comparing the results in this study, Ivermectin has little permanent effect than Doxycycline on female adult worms, the drug clears microfilaria from the skin and blood for a period of months. In this research work, Doxycycline had a maximal macro and microfilaricidal effect than CDTi and the plant extracts which was observed to have minimal therapeutic effect on subjects. This is in line with works done by [14,15,16,17], where their results showed sustained effects of six weeks Doxycycline treatment of *Onchocerca volvulus* depletion when compared to those who received treatment with Ivermectin alone. In a randomized clinical study conducted by [18,19], results showed that when Doxycycline was orally taken by subjects for three weeks, it significantly reduced the microfilarial load and was more effective in inducing microfilariaemia for up to two years when compared to monotherapy with Albendazole or Ivermectin. Evidence based scientific reports in this study lend credence to the value of Medicinal plants [20,21,22,23,24,25] and documented evidence suggested that *Morinda lucida* remains a very rich source of extract and chemical compounds with diverse bioactivities that are of therapeutic benefit to man including the treatment of Onchocercal infection as researched by previous authors. [26,27]. *Indigofera tinctoria* on the other hand contains secondary metabolites with highest amount of bioactive compounds used in the treatment of a wide

variety of ailments with little or no toxicity effects. Nevertheless, this does not justify its uses for the primary healthcare needs of indigenous populations across tropical Africa including these studied areas in Imo state Nigeria. Oral Doxycycline still demonstrated a higher efficacy compared to *M. lucida* and *I. tinctoria* even in low density infected areas of this study. This is in line with studies carried out by [28,29,30]; on the aqueous extract of *M. lucida* leaves, stem barks and roots as having more of anti-malarial effect, hypoglycemic properties [31,32,33], Cytotoxic effect by [34,35] and so many studies revealing its antibacterial, antispermatogenic, hepatotoxic, antihyperlipidemic, antioxidative potencies rather than antihelminthic properties. Many researchers also reported the therapeutic efficacy of *I. tinctoria* extract which offers an alternative source for developing a phytomedicine that may be used in treating filarial infections [36,37,38]

The results obtained from this study have also established the anti-Onchocerca activities of extracts of *M. lucida* and *I. tinctoria* as a possible new source for developing a phytomedicine or drug for the treatment of the onchocerciasis disease in the studied communities. These findings corroborate the report of [39,40]. This work justifies the use of these plants particularly in their crude form for treating onchocerciasis diseases.

CONCLUSION

Oral dose of Doxycycline was found to be the most effective treatment for Onchocercal infection in this study and this will enhance control, elimination and perhaps total eradication of macro- and microfilarial load in individuals suffering from the infection. Similarly, Doxycycline demonstrates higher efficacy and superiority in therapeutic effect as opposed to the widely accepted CDTi therapy and should thus be considered the most suitable and safest treatment regimen for Onchocercal infection. The two plant species used in this study (*Indigofera*

tinctoria and *Morinda lucida*) can be replaced with conventional methods of treating onchocerciasis infection in the studied communities. This study validates the appropriate use of these medicinal plants by traditional health practitioners in managing *Onchocerca volvulus* infection.

Declaration by Authors

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