

Research Article

Study of the Anthelmintic Properties of Some Extracts of *Vernonia Amygdalina* and *Ageratum Conyzoides* (Asteraceae) on the Parasite *Onchocerca Ochengi*

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Abstract

Like most countries in sub-Saharan Africa, Cameroon is affected by onchocerciasis. Commonly referred to as 'river blindness', it is a filariasis caused in humans by the parasite *Onchocerca volvulus*, which is transmitted to its host by a small fly belonging to the genus *Simulium*, with only the females being blood-feeding and transmitting the infectious larvae during their blood meals. Chronic infection causes itching and disfiguring lesions on the skin, as well as eye lesions that can lead to irreversible blindness. The current intervention strategy relies on mass drug administration (MDA), specifically ivermectin. Despite the efforts made to combat onchocerciasis, it remains a major public health issue and thus constitutes an obstacle to the socio-economic development of affected communities. Indeed, the absence of a macrofilaricide treatment, the inaccessibility of ivermectin for certain community groups, the prohibition of ivermectin for individuals co-infected with the filaria *Loa loa*, and the emergence of parasite strains resistant to the only available treatment slow down onchocerciasis elimination projects especially in Africa. Another alternative would then be to exploit the pharmacological properties of certain local medicinal plants that have proven effective in traditional medicine, in the treatment of parasitic diseases, particularly filariasis. The general objective of this work was to formulate anthelmintic molecules from local medicinal plants to effectively fight against onchocerciasis. The methanolic, ethanolic, acetonic, and ethyl acetate extracts of *Vernonia amygdalina* and *Ageratum conyzoides* were tested *in vitro* on the bovine parasite *Onchocerca ochengi*. Two ranges of concentrations were used: 0 to 300 µg/mL, then from 300 to 600 µg/mL in RPMI-1640. The results obtained from the tests showed that the methanolic and ethanolic extracts of *V. amygdalina* roots were the most effective with respective LC₅₀ of 68.52 ± 9.33 and 74.66 ± 3.72 µg/mL. Ivermectin used as a positive control revealed the best anthelmintic activity with a LC₅₀ of 17.782 ± 2.018 µg/mL.

Keywords

Plants, Drugs, Filariases, Ngaoundéré, Cameroon

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Received: 7 June 2025; Accepted: 23 June 2025; Published: 28 July 2025



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1. Introduction

Filariasis represents a group of endemic tropical diseases caused by viviparous nematodes called filariae [1]. These infestations are among the most distressing and disfiguring of parasitic diseases. It is estimated that around 1 billion people are at risk of contracting filariasis [9, 8]. However, they fall under the category of "Neglected Tropical Diseases (NTD)" simply because these infestations are restricted to tropical areas and are closely linked to poverty and social inequalities. They heavily afflict populations, particularly the most disadvantaged, marginalized, and isolated communities [22]. Although these infestations do not cause significant mortality and morbidity compared to other parasitic infestations, they nonetheless have a substantial but often less measurable effect [20]. Onchocerciasis is part of the NTDs and is classified as a category of chronic endemic diseases [2]. It is caused by a filarial nematode specific to humans, *Onchocerca volvulus*, which lives in the dermis and is transmitted to humans by a dipteran of the genus *Simulium*, whose larval habitats are located in fast-flowing watercourses, thereby increasing the risk of infestation among people living nearby, hence the common name "river blindness" [2]. Microfilariae cause intense inflammatory reactions, particularly when they die. From there, this inflammation can lead to skin lesions known as 'lizard skin' that are very debilitating, as well as ocular damage that can progress to complete irreversible blindness, depriving patients of their ability to work in the prime of their lives [3]. Affected individuals then experience low self-esteem and are victims of social isolation. Thus impoverished, villages heavily affected by this parasitosis suffer serious socio-economic consequences [16, 5]. The WHO estimates that among the 187 million people living in areas where there is a potential for transmission of this parasite, at least 17.7 million are infected and 1 million are blind or have severe visual impairment [17]. Cameroon is also one of the endemic countries as this disease is prevalent in all ten regions of the country with a national prevalence rate of about 30%; the prevalence rates in the Centre and Littoral regions being 50% and 60% respectively [12]. This high prevalence of onchocerciasis in these regions is likely linked to the favorable environment for the development of the vector. Onchocerciasis has thus become a significant obstacle to socio-economic development. While in the past nodulectomy and vector control were the methods used, the current intervention strategy relies on mass drug administration (MDA), namely ivermectin (Mectizan). However, since this medication has only a partial effect on the parasite (microfilaricidal), adult worms can reproduce and spread the disease. Another challenge is that millions of people in Africa are infested with loiasis, also known as loa-loa, which makes them intolerant to ivermectin. There is no effective treatment available for river blindness for these individuals [21]. In Cameroon, ivermectin has reportedly caused severe inflammatory reactions in indi-

viduals co-infected with the *Loa loa* parasite [21]. Moreover, the frequent use of ivermectin in the treatment of onchocerciasis and other helminthiases has inevitably led to the emergence of parasite strains resistant to the drug [10]. The combination of these factors therefore stimulates the search for alternatives or complementary solutions that may allow the control of this parasitosis. Bioactive plants with anthelmintic properties are now one of the main alternative or complementary solutions to the anthelmintics explored. After conducting ethnobotanical investigations with traditional healers, we were able to identify some medicinal plants used to treat helminthiases. The laboratory screening allowed us to retain *Ageratum conyzoides* and *Vernonia amygdalina*. The general objective of this work was to formulate anthelmintic molecules from local medicinal plants to effectively combat onchocerciasis.

2. Material and Methods

2.1. Description of the Study Area

Located in the north of the Adamawa region, with Ngaoundere as its capital, the Vina Division lies between 12.82725 ° and 14.76536 ° East latitude and between 6.52270 ° and 7.99075 ° North. It is bordered to the north by the North Region, to the west by Faro and Deo Division, to the south by Mbere and Djerem Divisions, and to the east by the Mbere Division and the North Region. Physically, the Vina Division has a very dense relief, with highlands and lowlands, notably the plain of Mbe. It has a dense hydrographic network. Most of the watercourses in the Adamawa Region, and in the whole country, have their sources in this division, such as the Vina, the Djerem, the Mbere, the Faro Deo and the Benoue. Administratively, the Vina Division is divided into 08 sub-divisions: the locality of Mbe to the north, that of Martab to the west, Nyambaka to the south, Bebel to the east, Ngan-ha to the north-east and finally Ngaoundere I, II and III at the centre (Bebel, Ngan-ha, Ngaoundere I, II, III and Nyambaka constituted the survey area, Figure 1). It has a cosmopolitan population, with all the country's ethnic groups living in harmony with the local populations, notably the foulbes, Mboum, Gbaya and Dii. The climate is tropico-sudanian. There are only two seasons: the dry season runs from November to April, followed by the wet season from May to October. Average annual rainfall ranges from 900 mm to 1500 mm, with the highest record of 1862.9 mm in 1956. Generally speaking, livestock rearing, agriculture and, to a lesser extent, trade remain the main activities of the people of Vina division, and these involves around 70% of the working population. Livestock farming and agriculture are practiced together, usually giving rise to farmer-grazier conflict between the various players. Due to its biophysical characteristics, the

Vina is an area entirely dedicated to livestock farming.

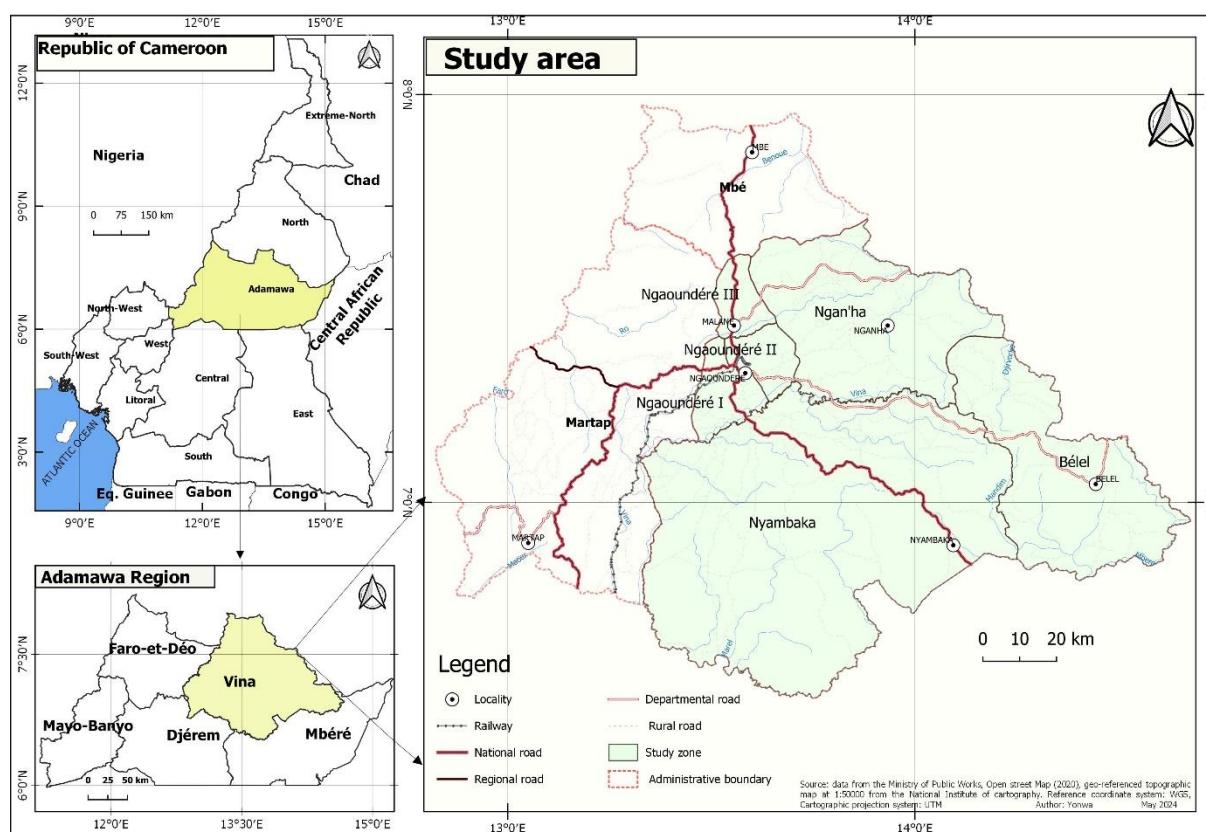


Figure 1. Location of the study area.

Source: Data from the Ministry of Public Works, Open street Map (2020), geo-referenced topographic map at 1:50000 from the National Institute of cartography. Reference coordinate system: WGS, Cartographic projection system: UTM.

2.2. Material

For this study, we used two types of material: the plant material made up of different parts of plants (Figures 1, 2, 3, and 4) and the plant material mainly consisting of portions of cow hides from which the nodules were extracted (Figure 5).



Figure 2. Leaf of *A. conyzoides* showing internal veins (4000 x 1920, Tecno Spark 3 Pro).



Figure 3. Roots of *A. conyzoides* (4000 x 1920, Tecno Spark 3 Pro).

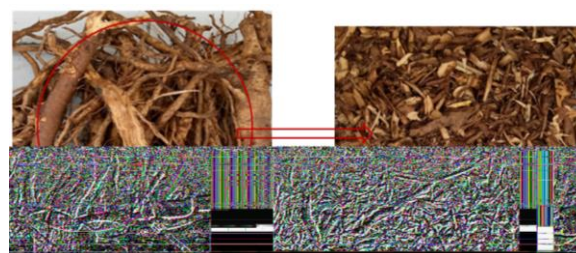


Figure 4. Fragments of roots of *V. amygdalina* (4000 x 1920, Tecno Spark 3 Pro).



Figure 5. Portion of cow's udder containing *onchocerciasis* nodules (4000 x 1920, Tecno Spark 3 Pro).

2.3. Methods

2.3.1. Ethnobotanical Survey

An ethnobotanical survey was conducted among 30 traditional healers in the city of Ngaoundéré (Adamaoua Region-Cameroon), Vina Division. The aim was to catalog through questionnaires all the medicinal plants used for the treatment of filariases in general, the method of extracting active principles, and the parts of the plants used.

2.3.2. Anthelmintic Tests

The anthelmintic efficacy of plant extracts was tested according to the protocol used by Borsboom *et al.* [4] (2003). We used 96-well plates and the RPMI-1640 solution which served as the nutritional medium for the parasite *Onchocerca ochengi*. Different concentrations of the plant extracts were used in vitro with the reference molecule being ivermectin (Mectizan). The culture plates containing varying concentrations of plant extracts in which the parasites were immersed were incubated in an incubator set at 37 °C for a duration of 48 hours. The mortality rate was evaluated using the colorimetric

test with the MTT tetrazolium salt (bromide of 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium).

2.3.3. Analysis and Processing of Data

The analysis of variance with one variable in repeated data was performed to compare the means of secondary metabolite contents of plant extracts and their LC₅₀ values, after proving the independence of residues, the normality of residues (Shapiro-Wilk test), and the homogeneity of variances (Levene's test on the median). To determine which means are statistically different from others, a post hoc analysis using the Tukey method was conducted; however, for the comparison of LC₅₀ values of extracts with ivermectin, the Dunnett test was applied. Principal component analysis (PCA) was applied to clarify the causal link between the quantities of secondary metabolites and the LC₅₀. The significance threshold was set at 5%, and the effects were noted as follows: not significant ($p > 0.05$) and significant ($p \leq 0.05$). Results were expressed as mean $\pm$ standard deviation. Statistical analyses and figures were generated using XLSTAT 2016.02.28451 software.

3. Results

3.1. Plants with Antifilarial Properties Recorded by Traditional Healers

The histogram in Figure 6 below illustrates the different plants with antifilarial properties recorded by traditional healers.

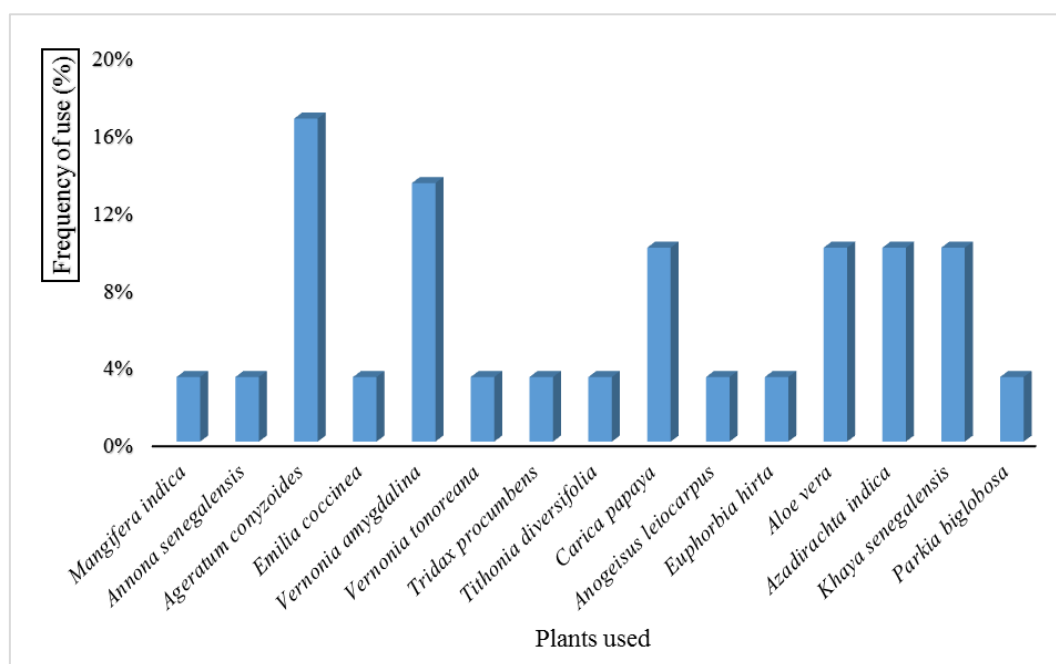


Figure 6. Frequency of use of the different listed plants.

3.2. Methods of Preparation of Extracts Chosen by Traditherapists

The pie chart below (figure 7) illustrates the proportions of extraction methods used by traditherapists during the different preparations of their anti-filarial remedies.

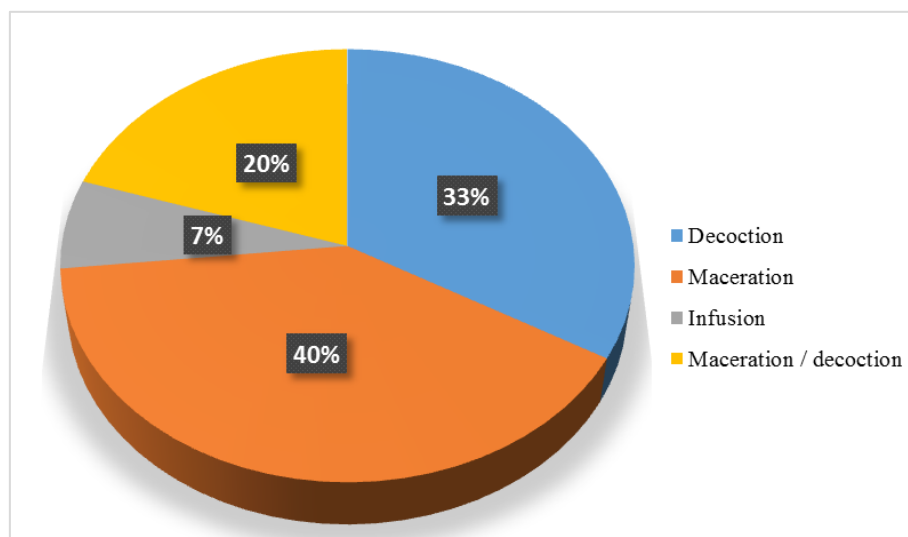


Figure 7. Preparation methods of the extracts chosen by the traditional therapists.

3.3. Parts of Plants Used by the Surveyed Traditional Therapists

The pie chart below (figure 8) shows the proportions of plant parts used by traditional therapists in the various preparations of their antifilarial remedies.

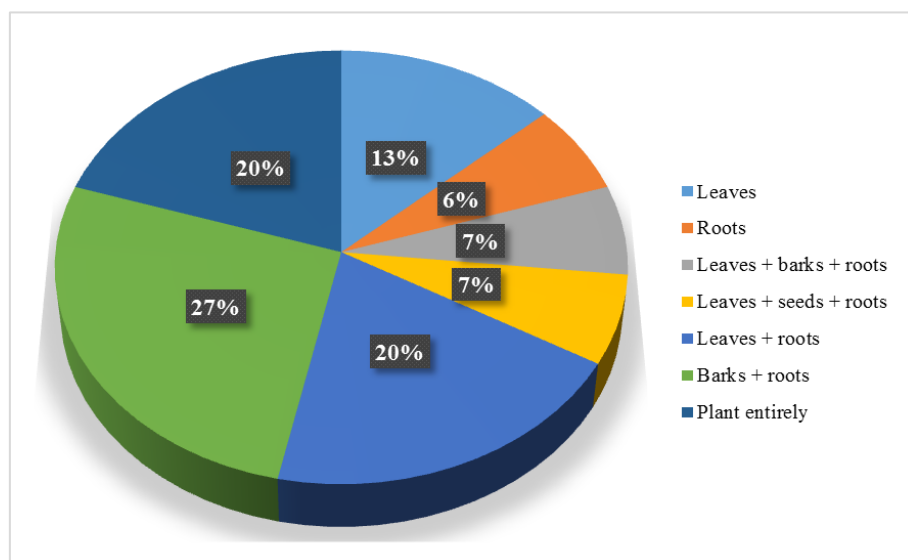


Figure 8. Parts of plants used by the surveyed traditional therapists.

3.4. Botanical Families of Plants Recorded with the Surveyed Traditional Therapists

The histogram below (figure 9) presents the different plant families to which the recorded species belong.

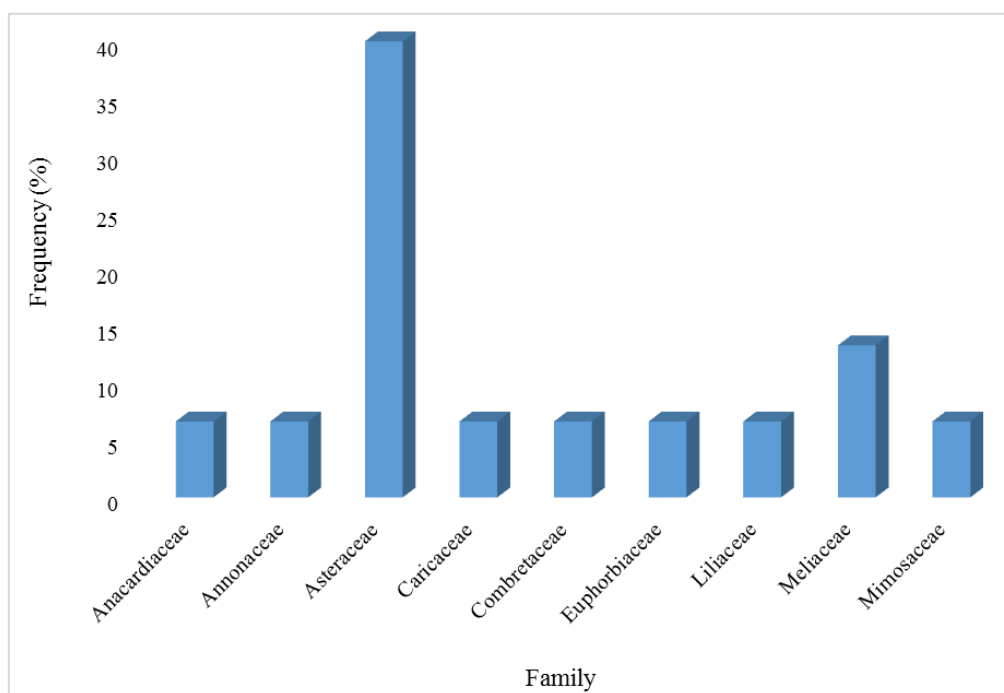


Figure 9. Botanical families of plants recorded with the surveyed traditional therapists.

3.5. Anthelmintic Tests

The results of the anthelmintic tests are presented in the figures below.

3.6. Results from the Leaves of *Ageratum conyzoides*

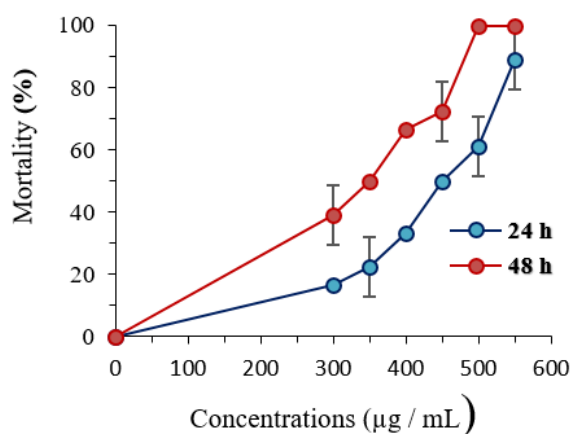


Figure 10. Ethanolic extract of *A. conyzoides* leaves.

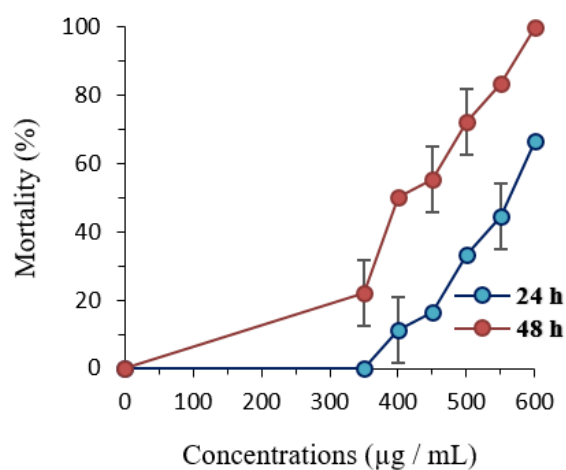


Figure 11. Ethyl acetate extract of *A. conyzoides* leaves.

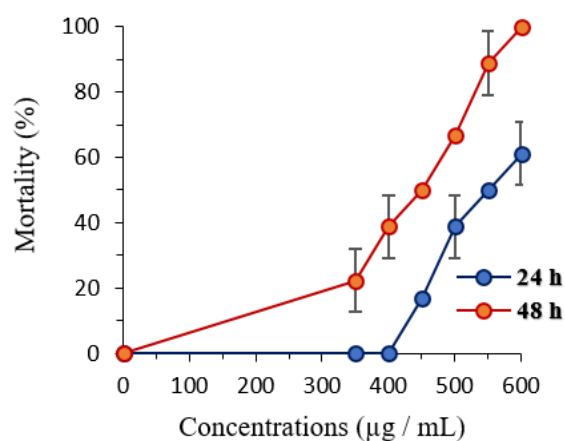


Figure 12. Acetic extract of *A. conyzoides* leaves.

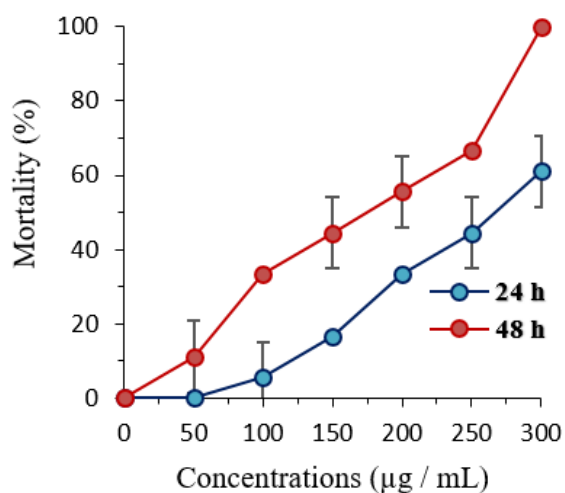


Figure 13. Methanolic extract of *A. conyzoides* leaves.

3.7. Results from the Roots of *Ageratum conyzoides*

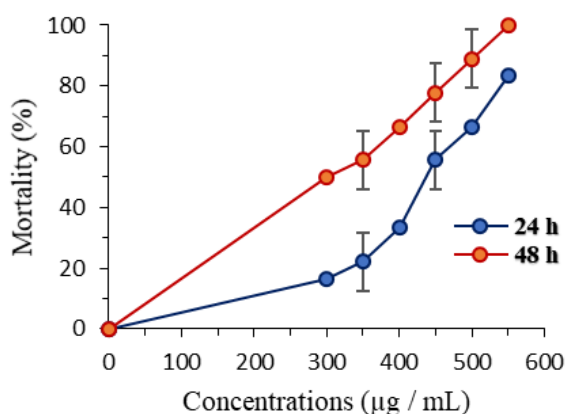


Figure 14. Ethanolic extract of *A. conyzoides* roots.

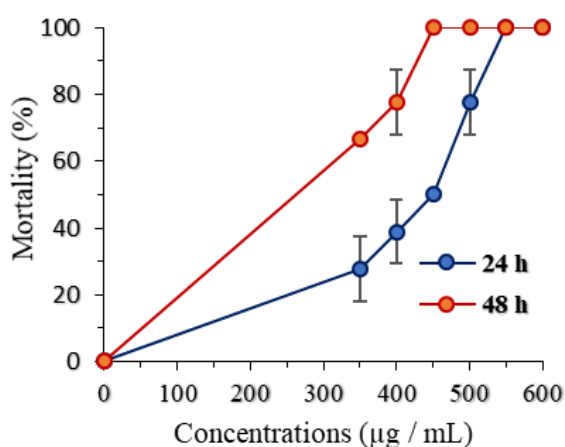


Figure 15. Acetonic extract of *A. conyzoides* roots.

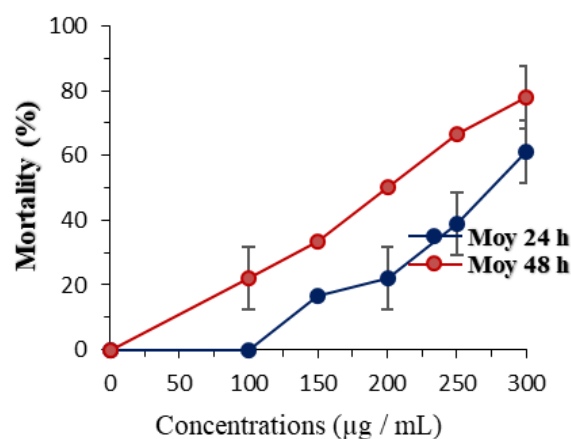


Figure 16. Methanolic extract of *A. conyzoides* roots.

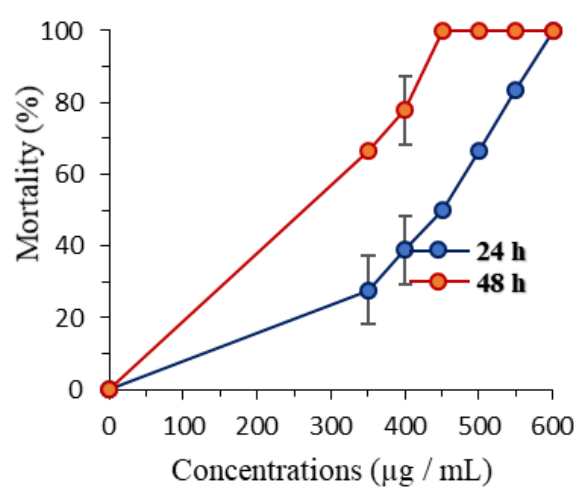


Figure 17. Ethyl acetate extract of *A. conyzoides* roots.

3.8. Leaves of *Vernonia amygdalina*

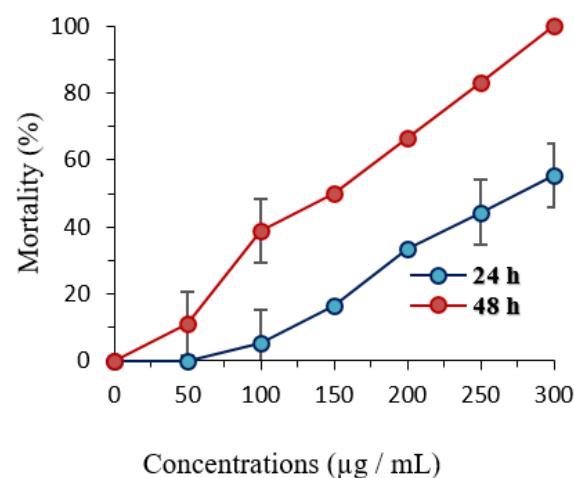


Figure 18. Ethyl acetate extract of *V. amygdalina* leaves.

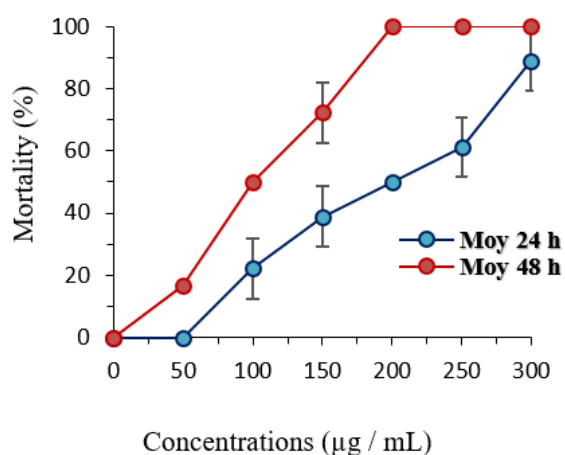


Figure 19. Methanolic extract of *V. amygdalina* leaves.

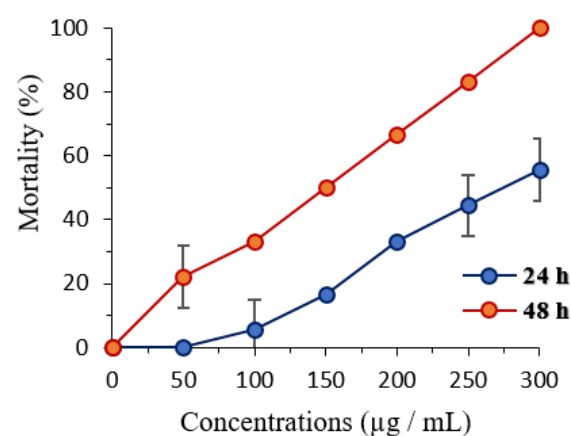


Figure 20. Acetic extract of *V. amygdalina* leaves.

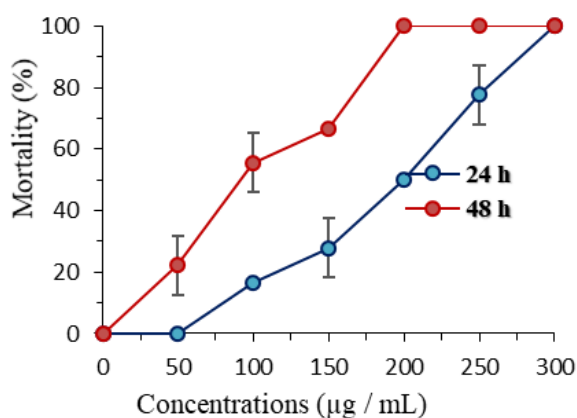


Figure 21. Ethanolic extract of *V. amygdalina* leaves.

3.9. Roots of *Vernonia amygdalina*

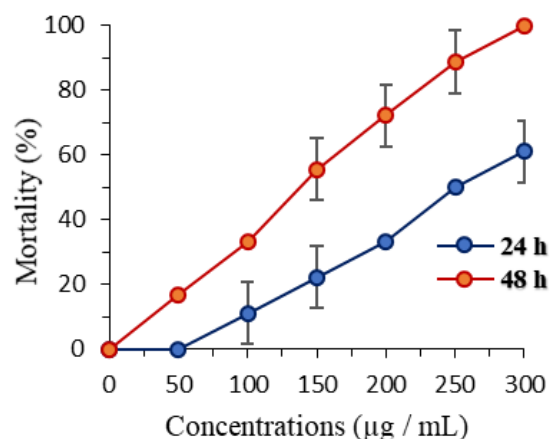


Figure 22. Ethyl acetate extract of *V. amygdalina* roots.

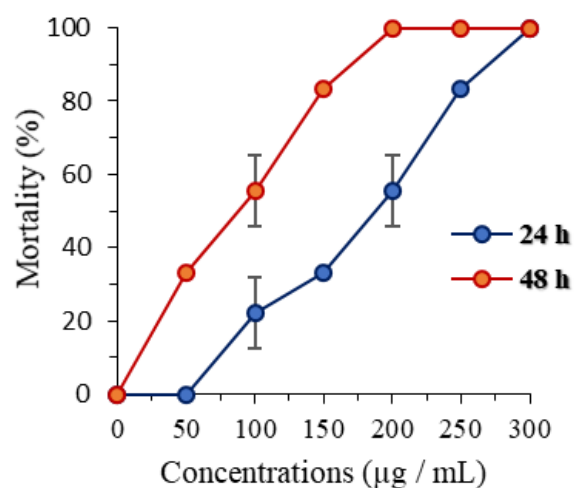


Figure 23. Ethanolic extract of *V. amygdalina* roots.

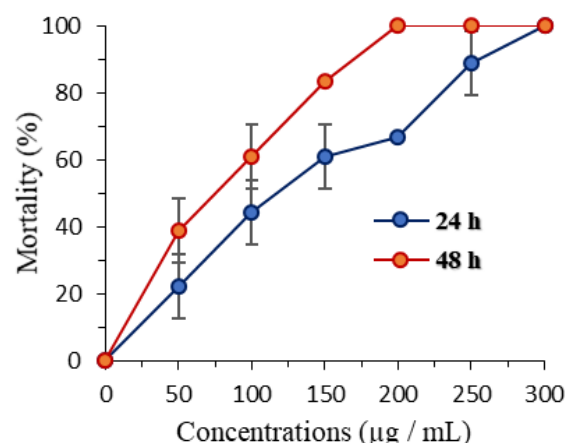


Figure 24. Methanolic extract of *V. amygdalina* roots.

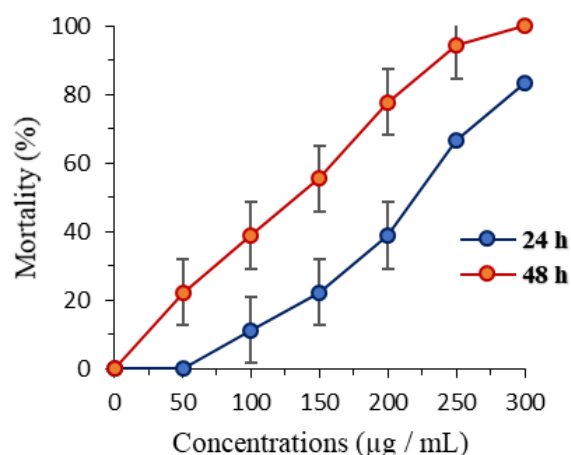


Figure 25. Acetonetic extract of *V. amygdalina* roots.

4. Discussion

At the end of the ethnobotanical surveys conducted among the traditional healers of the city of Ngaoundéré (Adamawa-Cameroon), we listed 15 plants with antifilarial properties. Based on the results obtained, *Ageratum conyzoides* and *Vernonia amygdalina* showed the highest frequency indices with 16.66% and 13.33% respectively. In the light of the literature, the botanical families mentioned by the respondents have been previously reported for their antifilarial properties. Among the 9 families identified, Asteraceae represents 40%. These results corroborate those of Kamoreng *et al.* [11], also reporting the use of members of Euphorbiaceae, Fabaceae, Rubiaceae, and Asteraceae, mentioning a predominance of Asteraceae. The ethnobotanical study reveals that the commonly used plant parts are respectively the roots, leaves, bark, and whole plant. Maceration is the most commonly used extraction method by traditional healers at 40%, followed by decoction (33.33%). Indeed, the extraction technique is a very important step in isolating and recovering the phytochemical compounds present in the plant material [18]. It is influenced by its chemical nature, the method used, the size of the studied sample, as well as the presence of interfering substances. Daoudi *et al.* [7] support this hypothesis by noting that the plant species, plant organ, drying conditions, the richness of each species in metabolites, and the nature of the solvent used for extraction affect the yield.

For the demonstration of the nematocidal activity of the extracts, we used the MTT colorimetric assay, which is an improvement over the motility/viability test. This test is based on the ability of living cells to reduce the yellow MTT into its metabolite, blue formazan (purple in color). The number of living cells after 48 hours of incubation in the presence or absence of the compounds to be tested or reference products is directly proportional to the intensity of the purple coloration. Thus, all purple-colored worms are considered alive while colored yellow-colored ones are considered dead worms [13]. This method has been used by several authors to highlight the nematocidal activity of extracts on the parasite *O. ochengi* [6,

14, 15]. After counting the number of dead and alive worms for each extract, only those extracts that produced 100% mortality of the parasite were selected. We thus eliminated plants which absolute anthelmintic activity exceeded 600 µg/mL. Consequently, we eliminated *Tridax procumbens* and *Euphorbia hirta*, leaving only *V. amygdalina* and *A. conyzoides*.

The results obtained show that the antifilarial efficiency of extracts and conventional molecules depends on the extraction solvent, concentration, and duration of incubation. The concentration-response profile of mortality (%) of adult males of *Onchocerca ochengi* subjected to different concentrations of leaf extracts of *A. conyzoides* showed after 48 hours that the ethanolic and methanolic extracts were the most effective with LC_{50} of 321.53 ± 9.65 µg/mL and 342.32 ± 9.62 µg/mL respectively. The ethyl-acetate and acetone extracts, on the other hand, were found to be less effective compared to the previous two with respective LC_{50} of 419.20 ± 9.2 µg/mL and 430.13 ± 10.11 µg/mL. On the other hand, after 24 hours of incubation, we can observe a slight difference in the trend. Indeed, the ethanolic extract is still more effective compared to the methanolic extract with respective LC_{50} of 431.64 ± 12.54 and 434.90 ± 5.51 µg/mL. However, in this case, the acetonetic extract proves to be more effective than the ethyl acetate extract with respective LC_{50} of 551.81 ± 14.78 and 553.90 ± 10.53 µg/mL.

Regarding the roots of *A. conyzoides*, the methanolic extract proved to be more effective than the ethanolic extract after 48 hours of incubation, with respective LC_{50} of 179.22 ± 5.00 and 302.52 ± 10.23 µg/mL. The acetonetic and ethyl-acetic extracts, on the other hand, showed the same efficiency with a LC_{50} of 335.71 ± 4.60 µg/mL. After 24 hours of incubation, the trend remained almost the same, with the only difference that the acetonetic extract was slightly more effective than the ethyl-acetic extract, with respective LC_{50} of 416.24 ± 8.68 and 427.42 ± 2.08 µg/mL ($p > 0.05$). These results corroborate those of Rhazi *et al.* [19] showing that the efficiency of an extract depends on the polarity of the extraction solvent.

The ethanolic extract of the leaves of *V. amygdalina* (LC_{50} 176.36 ± 10.01 µg/mL) showed after 24 hours of incubation a higher anthelmintic effectiveness than that of the methanolic extract (LC_{50} 181.68 ± 5.63 µg/mL) ($p > 0.05$). The ethyl acetate extract, on the other hand, was the least effective (LC_{50} 269.58 ± 8.88 µg/mL). We observe a considerable increase in effectiveness after 48 hours. The ethanolic, methanolic, acetonetic, and ethyl acetate extracts obtained LC_{50} values of 87.49 ± 6.26 , 92.14 ± 3.26 , 120.34 ± 9.20 , and 127.98 ± 4.03 µg/mL respectively.

5. Conclusion

Ultimately, this work was about finding an alternative treatment for filariasis to address the emergence of resistance to anthelmintics, which is causing deep concern in the medical field and highlighting an urgent need for new molecules. Human onchocerciasis (or river blindness) is a disease of the

eyes and skin caused by the parasitic worm *Onchocerca volvulus*, transmitted by flies of the *Simulium* species (black flies) that breed in fast-flowing waterways. Chronic infection causes itching and disfiguring skin lesions, as well as eye lesions that can lead to irreversible blindness. The limitations of ivermectin have led us to explore other avenues for the treatment of the disease. Local natural essences, specifically medicinal plants, offer great opportunities in the search for new molecules for antifilarial treatments. Ethnobotanical surveys conducted with 30 traditional healers in the city of Ngaoundéré allowed us to identify 15 medicinal plants with antifilarial properties. After screening, only two plants were selected. The extracts of *Vernonia amygdalina* and *Ageratum conyzoides* were tested *in vitro* to evaluate their anthelmintic potential on the adult stage of the bovine parasite *Onchocerca ochengi*. The results obtained from the tests showed that the methanolic and ethanolic extracts of the roots of *V. amygdalina* were the most effective with CL₅₀s of 68.52±9.33 and 74.66±3.72 µg/mL, respectively. Ivermectin used as a positive control revealed the best anthelmintic activity with a CL₅₀ of 17.782±2.018 µg/mL.

Abbreviations

MDA	Mass Drug Administration
MTT	Tetrazolium Salt (bromide of 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium)
NTD	Neglected Tropical Diseases
RPMI	Roosevelt Park Memorial Institute
WHO	World Health Organisation

Author Contributions

Djedoubouyom Name Elysee: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing

Abladam Darahalaye Elias: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing - original draft,

Nveikoueing Francis: Data curation, Formal Analysis, Software

Megnigieu Mimi Elodie: Resources, Writing - review & editing

Lassango Gassida Melanie: Resources, Writing - review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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