

In Vitro Study of the Antiparasitic Activity of *Vitellaria paradoxa* Gaertn (Sapotaceae) on the Parasite *Onchocerca ochengi*

Martinien Atakewang Djetoloum¹, Nadlaou Bessimbaye^{1*}, Mbaïnguam Mbailao²

¹Department of Biological and Pharmaceutical Sciences, Faculty of Human Health Sciences (FSSH), Laboratory of Research, Diagnostics and Scientific Expertise (LaboReDES), Unit of Toxicology, Pharmacology and Parasitology, BP 1117 N'Djamena/Chad
²Department of Biological Sciences, Faculty of Exact and Applied Sciences, Laboratory of Toxicology and Pharmacology, BP 1117 N'Djamena/Chad

DOI: [10.36347/sajb.2024.v12i01.002](https://doi.org/10.36347/sajb.2024.v12i01.002)

| Received: 27.11.2023 | Accepted: 04.01.2024 | Published: 09.01.2024

*Corresponding author: Nadlaou Bessimbaye

Department of Biological and Pharmaceutical Sciences, Faculty of Human Health Sciences (FSSH), Laboratory of Research, Diagnostics and Scientific Expertise (LaboReDES), Unit of Toxicology, Pharmacology and Parasitology, BP 1117 N'Djamena/Chad

Abstract

Original Research Article

Onchocerciasis is a parasitic disease that affects millions of animals and people around the world. A recipe based on *Vitellaria paradoxa* is used in traditional medicine against this disease in Chad. The objective of this study was to evaluate the antiparasitic activities of extracts from the roots, fruits, leaves and bark of *Vitellaria paradoxa* on the parasitic nematode *Onchocerca ochengi*, a model similar to *Onchocerca volvulus* used in the research of control drugs against onchocerciasis. The identification of parasites and the hydroethanolic extracts of the different planar organs were carried out at the Laboratory of Research, Diagnostics and Scientific Expertise (LaboReDES) of the Faculty of Human Sciences, University of N'Djamena using the methods of Ndjonka *et al.*, 2012. The extracts of this plant were tested at different concentrations (0.1; 0.2; 0.3; 0.4 mg/mL) in the RPMI-1640 culture medium. The antiparasitic activity was noted according to the mortality rate after 72 hours of incubation of *Onchocerca ochengi* at 37°C. The LC50 values were then graphically determined at the end of this incubation. They were 0.05 mg/mL for the roots; 0.06 mg/mL for fruits; 0.05 mg/mL for the leaves and 0.06 mg/mL for the bark. These results confirm the effectiveness of *Vitellaria paradoxa* used in traditional medicine to treat parasitosis. This plant also has the same effects as ivermectin. The *V. paradoxa* root test on microfilariae shows an LC50 value of 0.05 mg/mL in 24 hours of incubation. This study showed that the hydroethanolic extracts of the bark of *Vitellaria paradoxa* had antiparasitic activities, allowing the development of a new molecule active against these parasitic infections and also the extracts of this plant could be used as alternatives to treat infections in case of parasite resistance to ivermectin.

Keywords: *Vitellaria paradoxa*, *Onchocerca ochengi*, antiparasitic activity, bovine, Chad.

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Onchocerciasis is a cutaneous-dermal helminthiasis caused by *Onchocerca volvulus* transmitted to humans by an insect vector of the genus *Simulium* (WHO 2011). During a blood meal they transmit microfilariae. The adult worm is either free in the dermis or trapped in a fibrous nodule called "onchocercoma".

Human onchocerciasis exists in tropical regions and occurs in 31 African countries. It is also prevalent in Yemen, in 6 countries in Central and South America with 15.66 million people infected worldwide, including 1.15 million cases of blindness and 220 million people exposed, 99% of whom live in Africa (WHO, 2022).

It often manifests itself as subcutaneous nodules whose size varies from a few millimeters to a few centimeters. The number of nodules on the same subject is very variable and strongly depends on the age and intensity of their infection (Djafsia *et al.*, 2018). It also manifests itself by ocular symptoms considered to be the most serious signs of onchocerciasis. These symptoms are due to the presence of dead or living microfilariae in the eye (Kalmobé *et al.*, 2017).

In Chad, onchocerciasis is endemic in all southern provinces and in Chari Baguirmi. Epidemiological data on onchocerciasis from July 25, 2013 shows that out of 31 villages surveyed in the south of the country, we note a prevalence varying between 0.20% and 3.4%. The regions most affected are: Chari

Baguirmi, Tandjilé, Mayo Kebbi, Mont de Lam, the two Logones (eastern and western) and Middle Chari (WHO, 2013).

Added to the health problem is the socio-economic problem. For this reason, different control programs have been developed. Thus in 1974, the International Community created the Onchocerciasis Control Program (OCP) which extended over West Africa and whose main strategy was based on the control of the vector by the application of insecticides and larvicides in high-flow rivers. The African Onchocerciasis Control Program (APOC) created in 1995 aimed to discover an effective filaricide agent. It covers 19 countries on the African continent and its strategy was Community Directed Ivermectin Treatment (CDTI) (Eisenbarth *et al.*, 2016).

Added to the health problem is the socio-economic problem. For this reason, different control programs have been developed. Thus in 1974, the International Community created the Onchocerciasis Control Program (OCP) which extended over West Africa and whose main strategy was based on the control of the vector by the application of insecticides and larvicides in high-flow rivers. The African Onchocerciasis Control Program (APOC) created in 1995 aimed to discover an effective filaricide agent. It covers 19 countries on the African continent and its strategy was Community Directed Ivermectin Treatment (CDTI) (Eisenbarth *et al.*, 2016).

Given the diversity of the Chadian flora, as well as the limited number of local plants that have been the subject of clinical investigation, it seemed important to us to study some of these plants used in traditional medicine to treat various pathologies, in general, and parasitosis in particular. This is how *Vitellaria paradoxa*, renowned for its traditional therapeutic effects, was chosen. This plant has been the subject of several scientific studies having shown its various activities, such as butter used for the treatment of dermatological diseases and sore throats; leaves and bark to treat stomach aches, headaches, eye problems; the roots in diarrhea treatments (Dikti *et al.*, 2017, Bayala *et al.*, 2018).

A livestock country, Chad has 93.8 million livestock. It is made up of ruminants such as goats (32.5%), sheep (28.2%), cattle (32.5%) and camels (6.8%) according to the general livestock census of the Ministry of Livestock and Animal Production and the Food and Agriculture Organization of the United Nations in 2018.

The objective of this work was to evaluate the antiparasitic activity of *Vitellaria paradoxa* extracts on the parasitic nematode *Onchocerca ochengi*, a model similar to *Onchocerca volvulus* used in the research of drugs to combat onchocerciasis. By setting up an

antiparasitic drug, we will provide an effective solution to the fight against animal and human onchocerciasis.

MATERIAL AND METHODS

Type and Period of Study

This was a prospective and experimental study which lasted three months (3 months), going from January 1, 2023 to March 31, 2023 including for the collection of the different organs of the plants, transport and preparation at the Research and Diagnostic Laboratory and Scientific Expertise (LaboReDES) and two months for the identification of parasites, hydroethanolic extracts and parasitic activity tests.

Biological material

Skin samples containing nodules of *O. ochengi* are taken by collecting portions of skin at the upper level from cattle slaughtered at the Farcha refrigerated slaughterhouse in the 1st Arrondissement of N'Djamena and transported for the identification of parasites at LaboReDES.

It also consists of the roots, leaves, bark and fruits of *V. paradoxa* harvested in January 2013 in Goré in the Logone Orientale region (southern Chad). The collection of samples was carried out thanks to the support of certain traditional practitioners present in the harvesting areas. The identification and confirmation of the species was made at the herbarium of the Institute of Research for Development (IRED).

Methods

Preparation of plant extracts

The plant materials (leaves, fruits, roots and fresh bark) were dried in the laboratory (LaboReDES) at room temperature (25°C) and pulverized into a fine powder for extraction by a Moulinex brand from Binatone. Several extracts were prepared from the powder obtained. The procedure was carried out using ethanol-water (70:30 v/v) and an organic solvent such as petroleum ether.

Fat extraction

The fat from the powders of the different plant organs was extracted with petroleum ether. So to 400g of powders of each extract we added 2L of petroleum ether for 24 hours.

Hydroalcoholic extraction

50g of powder from each part of the plant were left to macerate in 500 ml of 70% ethanol for 48 hours. The mixture was centrifuged at 3500 x g for 10 minutes, then the supernatant was filtered using filter paper (5891 Black Ribbon, ashless) from the company Schleicher and Shuell in an Erlenmeyer and put in the oven at 50°C. The powder obtained was weighed, and 0.5g of this powder was dissolved in a solution containing 1045µL of DMSO and 3955µL of distilled water. This mixture was homogenized by vortex, placed in a bottle tube and kept at +4°C away from light in order to be used (Ndjonka *et*

al., 2012). This extract thus constituted the stock solution with a concentration of 100 mg/ml for our samples. These plant extracts gave different yields including 15% for the roots, 18% for the fruit, 22% for the leaves and 18% for the bark. For the preparation of ivermectin which constitutes our positive control, 1000 μ g was dissolved. This medication was then diluted with RPMI to obtain a solution with a concentration of 1000 μ g/ml. The negative control consisted of 0.0001 μ L of DMSO mixed with 1999.99 μ L of RPMI.

Nodule Sampling

The skins of cows with nodules on the udders were paid for at the Farcha refrigerated slaughterhouse and then taken to the laboratory. These portions were washed with distilled water then disinfected with 70% ethanol to avoid any source of contamination. Onchocercomas or nodules were isolated from the skin using a scalpel fitted with a scalpel blade by making a slight tear on the inner side of the skin. The isolated

nodules were placed in a freshly prepared PBS solution (Ndjonka *et al.*, 2018).

Dissection of nodules and isolation of male *O. ochengi*

A nodule is grasped between the thick forceps, a small incision in the nodule wall and slight pressure on the nodule causes the worms to emerge. From then on, the separation of males and females was done using fine forceps and a mounted needle. Males who were more mobile than females were collected using fine forceps and then placed in a sterile PBS solution (Ndjonka *et al.*, 2018). Throughout the dissection, all material is cleaned using toilet paper soaked in 70% ethanol to avoid any bacterial infections (Ndjonka *et al.*, 2018).

Cultivation of *Onchocerca ochengi*

After isolation, the live worms were brought under the hood (previously disinfected with UV and 95% ethanol to avoid any source of contamination) where we washed 3 times in PBS then 2 times in RPMI which constitutes our culture medium to which antibiotics (Penicillin and streptomycin) are added.

Table 1: Concentrations and volumes of DMSO in the culture medium containing the plant extracts

	Roots	Fruits	Leaves	Barks
C(mg/mL)	0.4	0.4	0.4	0.4
CDMSO in the tubes	0.08%	0.08%	0.08%	0.08%
Equivalent VDMSO	8	8	8	8
V _{RPMI} (μ L)	1992	1992	1992	1992
Number of worms	6	6	6	6

C = concentration; V = volume; DMSO = dimethylsulfoxide; RPMI = Roswell Park Memorial Institute medium

Pest test of the plant on adult *O. ochengi* worms

To carry out these tests, we carried out a screening with small, medium and large concentrations. The concentrations after 24 hours which killed all the worms are revised downwards and those which did not kill them are revised upwards. The adult worms of *O. ochengi* (6 worms/well) were incubated following the protocol of (Ndjonka *et al.*, 2018) at different concentrations of extracts from the parts of the plant used (0.1; 0.2; 0.3; 0.4 mg/mL) for all parts. In each well of the 24 plates, 2mL of RPMI is introduced to which a volume corresponding to each volume of the concentrations of our samples is added. The positive controls were carried out at ivermectin and negative controls with DMSO (0.0053%). Three tests were the subject of our work for each series of four

concentrations; the worms are incubated at 37°C and mortality is determined after 24 h, 48 h and 72 h (Ndjonka *et al.*, 2018).

RESULTS

Geographical location of the study area

The locality of Goré is located approximately 600 km south of N'Djamena and borders the Central African Republic (CAR). The different parts of the plant were collected in the Logone Oriental province located in the south of Chad, precisely in the locality of Goré, Department of Nya-Pendé. Rich in its flora variabilities, the town of Goré has a significant quantity of *V. paradoxa* used in the treatment of bacterial and parasitic diseases.

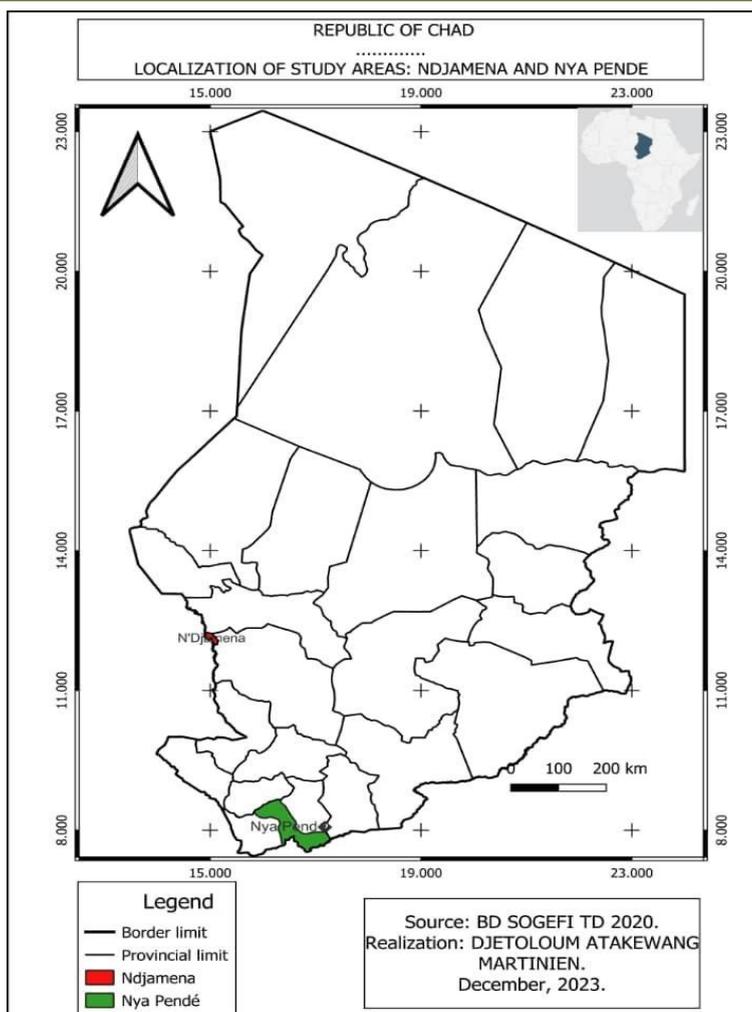


Figure 1: Presentation of the plant harvest area on the map of Chad

Extraction yields

After evaporation of the extracts, the pastes were obtained and weighed. The mass of the paste

obtained was divided by that of the powder initially weighed and multiplied by 100. This result obtained is the yield.

Table 2: Yields of extracts from the roots, fruits, leaves and bark of *Vitellaria paradoxa*

Plant	Excerpts	Yields
<i>Vitellaria paradoxa</i>	Roots	15%
	Fruits	18%
	Leaves	22%
	Barks	18%

The DMSO test on male *Onchocerca ochengi* worms

After their isolation, the male *Onchocerca ochengi* worms were cultured in the presence of 1% DMSO. No mortality was reported after 72 hours; which means that DMSO has no effect on worms.

Preliminary tests on *Onchocerca ochengi*

After their isolation of adult worms, preliminary studies made it possible to determine the optimal lifespan of adult male worms of *Onchocerca ochengi* in a culture medium consisting of RPMI-1640 containing penicillin/streptomycin antibiotics (200 IU of penicillin and 200 µg/mL of streptomycin per 200 mL of

medium). The worms obtained after dissection of nodules were incubated at 37°C. For 6 worms cultured in 2 mL of RPMI-1640 medium, 3 repetitions were carried out, the average of which was calculated (Table 1).

The mortality rate was 0% until the sixth day of culture. The first dead worms were observed on the seventh day of incubation (33.33%). This mortality increased and reached 83.33% on the ninth day. On the tenth day of culture, 100% mortality was noted. We can conclude that in RPMI-1640 culture medium, *Onchocerca ochengi* can live for 6 days.

Table 3: Percentage mortality rate of *Onchocerca ochengi* in RPMI culture medium

Mortality (%)	0	0	0	0	0	0	33.33	66.67	83.33	100
Day	1	2	3	4	5	6	7	8	9	10

Effects of ethanolic extracts of *Vitellaria paradoxa* on adult worms of *Onchocerca ochengi*

In this present study, we tested the anthelmintic activity of the ethanolic extract of the roots, fruits, leaves and bark of *Vitellaria paradoxa* on the bovine parasitic nematode *Onchocerca ochengi*. These extracts showed inhibitory and even lethal activity at different concentrations. Worm mortality was not only a function of time and part of the plant but also of various concentrations (0; 0.1; 0.2; 0.3; 0.4 mg/mL). The 5 concentrations were placed in culture tubes each containing 6 adult worms (Ndjonka *et al.*, 2021). The concentrations which killed half of the worms (LC50)

were determined graphically using Excel software after drawing the mortality curves.

Effects of *Vitellaria paradoxa* roots on *Onchocerca ochengi*

The LC50 of 3 repetitions of tests of different concentrations carried out on *O. ochengi* present different values after 72 hours of incubation at 37°C. These mortality curves for worms incubated at 37°C allowed us to bring out the LC50 at 24 h, 48 h and 72 h which are respectively 0.22; 0.12 and 0.05 mg/mL. We see 100% mortality at 24 and 48 hours at 0.4 mg/mL and at 72 hours at 0.1 mg/mL (Figure 2).

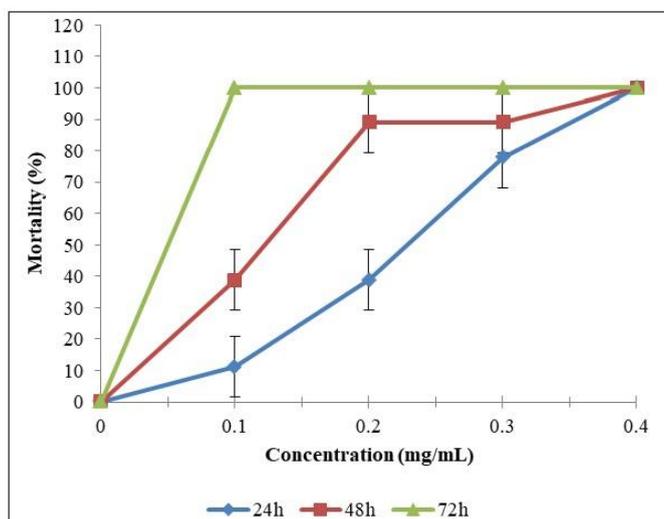


Figure 2: Curve showing the effects of *Vitellaria paradoxa* root extract on *O. ochengi*

Effects of *Vitellaria paradoxa* fruits on *Onchocerca ochengi*

The ethanolic extract of *V. paradoxa* fruits had considerable effects on the worms and the mortality rate reached 100% after an incubation of 48h and 72h at 37°C

with respective concentrations (0.2 and 0.4 mg/mL). On the other hand, this mortality is 77.78% in 24 hours of incubation at a concentration of 0.4 mg/mL. The LC50 value is 0.29 mg/mL in 24 h, 0.19 mg/mL in 48 h and 0.06 mg/mL in 72 h (Figure 3).

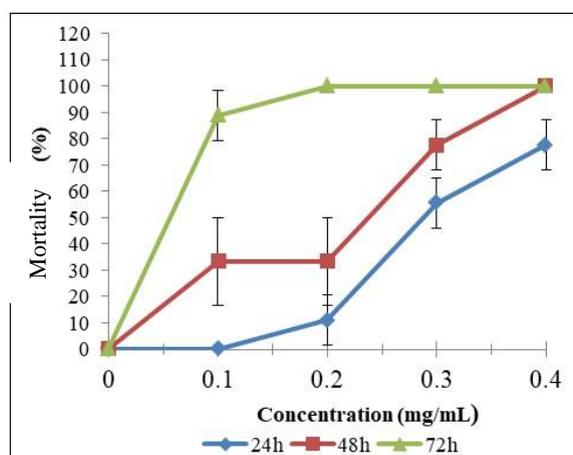


Figure 3: Curve showing the effects of *V. paradoxa* fruit extract on *O. ochengi*

Effects of *Vitellaria paradoxa* leaves on *Onchocerca ochengi*

For this extract we note that the mortality of male adults of *O. ochengi* is 55.56% in 24 hours of incubation at a concentration of 0.4 mg/mL. It reaches 100% in 72 hours at a concentration of 0.2 mg/mL. This rate then increases gradually over the hours and reaches

100% death after 72 hours of incubation at a concentration of 0.2 mg/mL. This mortality shows that the leaves of *V. paradoxa* have nematotoxic properties with LC50 values of 0.30mg/mL, 0.17mg/mL and 0.05mg/mL respectively in 24h, 48h and 72h of incubation at 37° vs.

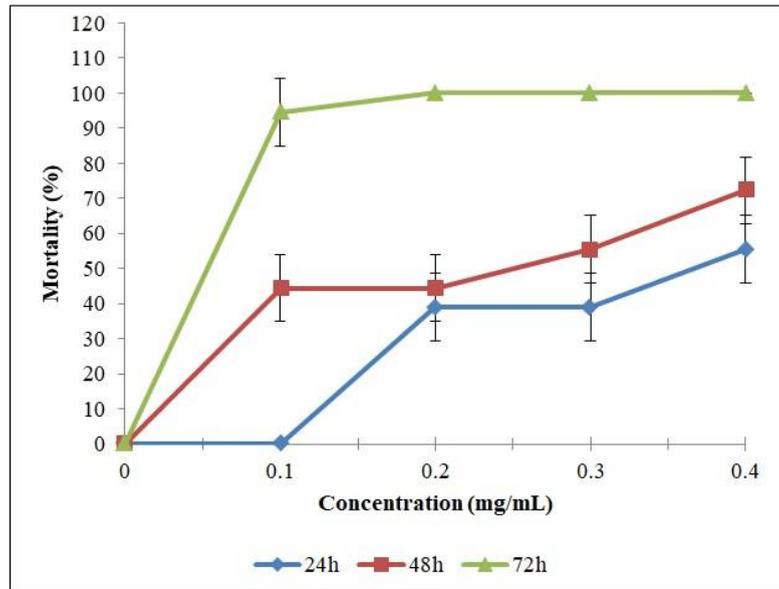


Figure 4: Curve showing the effects of *Vitellaria paradoxa* leaf extract on *O. ochengi*

Effects of *Vitellaria paradoxa* bark on *Onchocerca ochengi*

The averages of 3 repetitions of extract tests carried out on *O. ochengi* are shown in figure (20). The mortality curves of the worms incubated at 37°C

revealed the LC50 at 24 h, 48 h and 72 h which were respectively 0.27; 0.17; 0.06 mg/mL. A mortality of 66.67% was observed at 0.4 mg/mL in 24 hours. This rate gradually increases and reaches 100% with a concentration of 0.3 mg/mL after 72 hours.

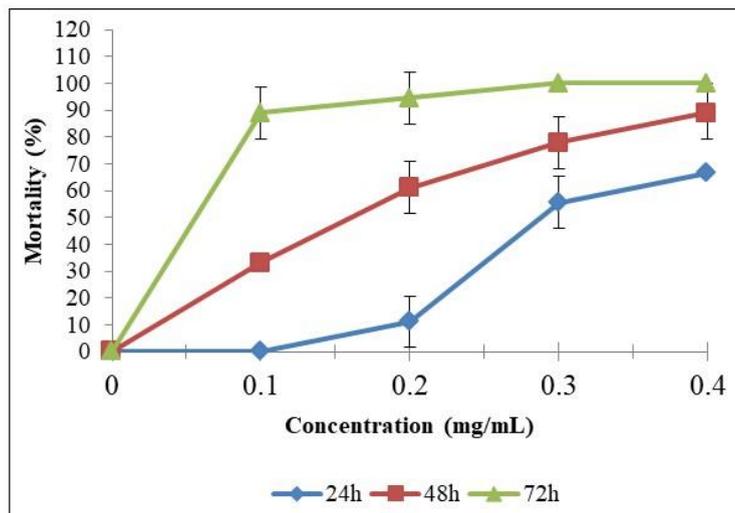


Figure 5: Curve showing the effects of *Vitellaria paradoxa* bark extract on *O. ochengi*

Effects of *V. paradoxa* roots on the parasite *Onchocerca ochengi*

In this study, it is important to test the anthelmintic activity of the most effective extract on *Onchocerca ochengi* microfilariae. Tests of these

different extracts on microfilariae have shown that the most effective part is the root. The ethanolic extract of this root is tested at different concentrations (0, 0.1; 0.2; 0.3; 0.4 mg/mL). The mortality of microfilariae was a function of time and these dependent concentrations. The

concentrations which killed half of the worms (LC50) were determined graphically after drawing the mortality curve considering the average of 3 repetitions. The average LC50 of 3 repetitions of tests of this extract

carried out on these microfilariae presents a value of 0.05 mg/mL using the Excel software after 24 hours of incubation at 37°C. This mortality rate is 100% with a concentration of 0.1 mg/mL after 24 hours.

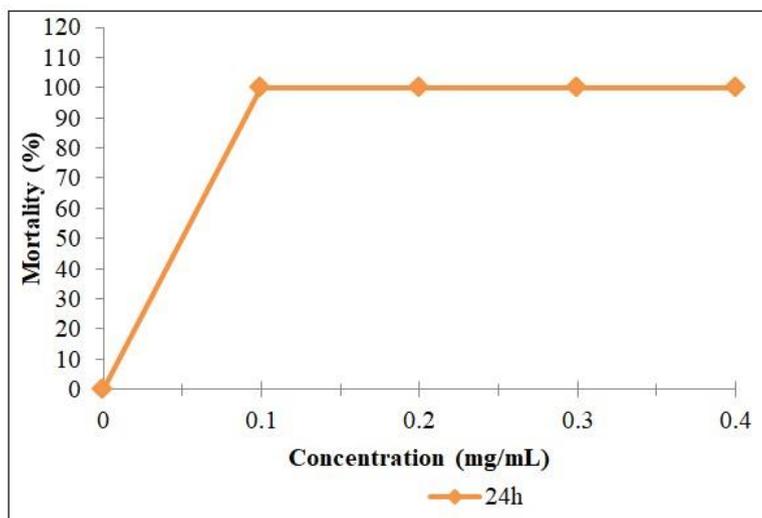


Figure 6: Curve showing the effects of *Vitellaria paradoxa* root extract on *O. ochengi* microfilariae in 24 hours
Effect of ivermectin on adult males of *Onchocerca ochengi*

The effects of ivermectin at different concentrations (0; 0.1; 0.3; 0.4, 0.5 mg/mL) on the mortality of *Onchocerca ochengi* in the RPMI-1640 culture medium are illustrated in the figure (22) at time slots of 24 hours, 48 hours and 72 hours. Male adults of

O. ochengi in the presence of ivermectin have a mortality rate of 76% after 24 hours of incubation at a concentration of 0.5 mg/mL. This mortality is 100% in 48 hours at 0.5 mg/mL and 72 hours with the same concentration. The LC50 values are 0.37mg/mL, 0.2mg/mL and 0.05mg/mL for 24 h, 48 h and 72 h respectively.

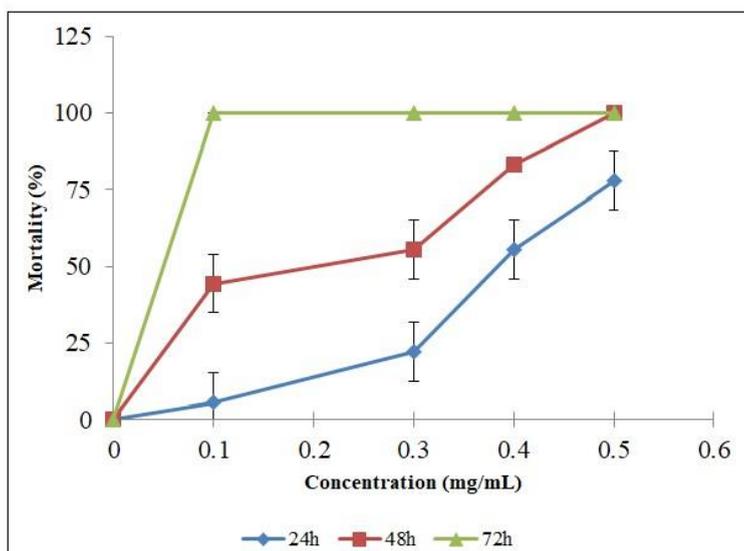


Figure 7: Curve showing the effect of ivermectin on adult males of *O. ochengi*
Comparative effects of plant extracts on *Onchocerca ochengi*

We notice that all the LC50 of the plant are less than 1 mg/ml and this shows the effectiveness of the small concentration of our extracts used against this parasitic nematode *O. ochengi*. The results obtained from

the LC50s after 72 hours of incubation at 37°C were the subject of a statistical analysis (ANOVA), which showed that the difference between the extracts is not statistically significant ($p = 0.05$). This difference is still not significant at the 5% threshold when we compared them to ivermectin.

Table 4: Comparison of LC50 of ethanolic extracts of *Vitellaria paradoxa* on male adults of *O. ochengi* after 72 hours of incubation

Plant LC50 mg/ml			
Excerpt	24h	48h	72h
Roots	0.22 ± 0.02 ^{ns}	0.12 ± 0.02 ^{ns}	0.05 ± 0.02 ^{ns}
Fruits	0.29 ± 0.02 ^{ns}	0.19 ± 0.08 ^{ns}	0.06 ± 0.01 ^{ns}
Leaves	0.30 ± 0.10 ^{ns}	0.17 ± 0.12 ^{ns}	0.05 ± 0.01 ^{ns}
Barks	0.27 ± 0.06 ^{ns}	0.17 ± 0.03 ^{ns}	0.06 ± 0.01 ^{ns}

P > 0.05; non-significant difference between the extracts of *V. paradoxa* on *O. ochengi* in 72 hours of incubation.

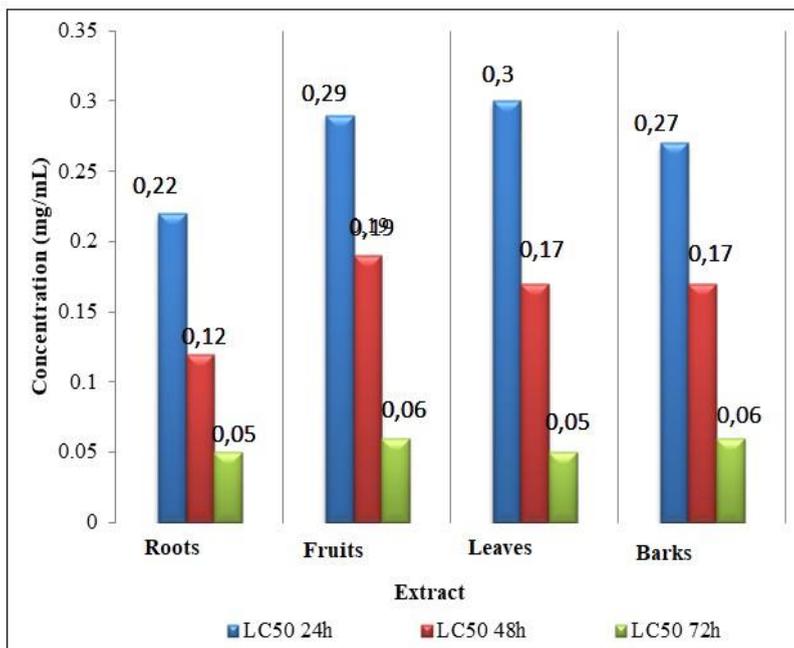


Figure 8: Comparative effects of ethanolic extracts of *V. paradoxa* on *O. ochengi* after 72 h

Comparative effects of plant extracts and the positive control on *Onchocerca ochengi*

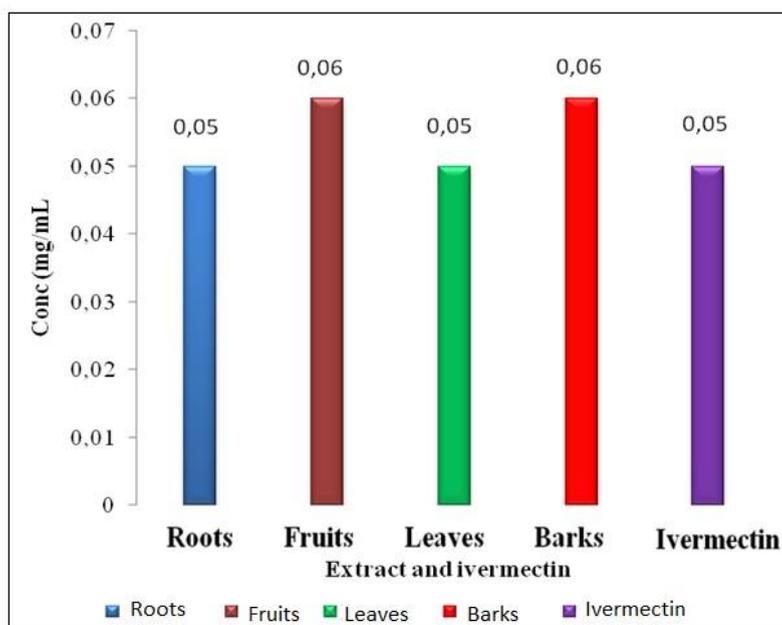
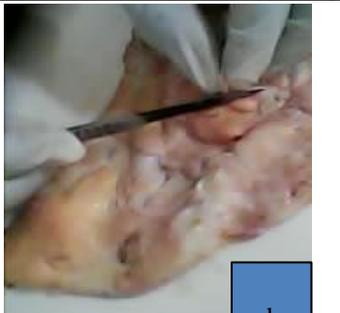
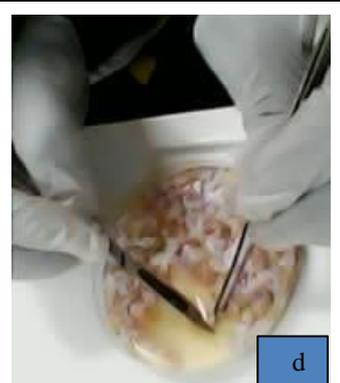


Figure 9: Comparative effects of *V. paradoxa* extracts and ivermectin on *O. ochengi*
p>0.05; non-significant difference between ivermectin and the different extracts

The analysis of variance (ANOVA) of our data allowed us to have the results of the LC50 of the plant extracts and ivermectin (reference product used for the fight against onchocerciasis). We note that all LC50s are less than 1 mg/mL. Which shows the effectiveness of the small concentration of our extracts used against this parasitic nematode *O. ochengi*.

This analysis of variance showed us that these results obtained from LC50 at the end of 72 hours of incubation at 37°C of the *V. paradoxa* extracts are not statistically significant between them and between ivermectin at the 5% threshold.

Table 5: The biotechnological steps of isolating *O. ochengi* and collecting and preparing *V. paradoxa* organs

1	<p>a: skin containing nodules</p> <p>b: nodule extraction</p> <p>c: isolated nodules</p>			
2	<p>d: nodule dissection</p> <p>e: roots</p> <p>f: leaves</p>			
4	<p>g: barks</p> <p>h: fruits</p>			

(Source: Djetoloum *et al.*, 2023)

DISCUSSION

During our studies, extracts from different parts of *V. paradoxa* showed antifilarial activity with LC50 values < 1 mg/mL. After 72 hours of incubation at 37°C, 100% mortality of worms was obtained at a concentration of 0.1 mg/mL with root and leaf extracts. On the other hand, this mortality rate reaches 88.89% with those of fruits and peels. Le test au DMSO n'a par contre entraîné aucune mortalité des mâles adultes de *O. ochengi* après 72 h d'incubation. These results are

similar to those of (Kalmobé *et al.*, 2017) concluding that the low concentration of DMSO (1%) present in the solution does not lead to worm mortality.

In the presence of ivermectin, the mortality of worms in the culture medium was considerably increased depending on the concentrations with an LC50 of 0.05 ± 0.026 in 72 hours. These results are similar to those of (Amina *et al.*, 2023) with an average LC50 of 0.07 ± 0.018.

The ANOVA test showed that the results of the ethanolic extract of different parts of *V. paradoxa* are comparatively insignificant between it and ivermectin. These results show that the different parts of the plant are as effective as ivermectin and act in the same way. The ethanolic extract of the roots had a greater influence on the mortality of male adults of *O. ochengi* compared to the other extracts. This influence was demonstrated by (Kalmobé *et al.*, 2017) on the low activity of dry leaves compared to roots observed in in vivo work on gastrointestinal nematodes in small ruminants (*Oesophagostomum*, *Haemonchus*, *Trychostrongylus*). It appears from this work that the anthelmintic properties of *V. paradoxa* are due to the presence of compounds such as saponins, tannins, alkaloids and flavonoids (Narhari *et al.*, 2015). In nematodes, these compounds cause death by paralysis (Narhari *et al.*, 2015).

Phytochemical tests of *V. paradoxa* carried out in previous work showed the presence of certain chemical families that would act as an anthelmintic. These include compounds such as tannins, flavonoids and saponins (Ndjonka *et al.*, 2017). The influence of the roots would be due to its high saponin content which would have acted on the permeability of cell membranes in order to continue its action on the nervous system and muscular function. It would have acted in particular by inhibiting neurotransmission (Aranzazu *et al.*, 2012). The tannins would react directly with the surface proteins of *O. ochengi*, causing physiological dysfunction in the nematode such as mobility and absorption of nutrients, which would cause the death of the worm (Kalmobé *et al.*, 2023). The work of Ndjonka *et al.*, (2012) showed the anthelmintic activity of *Anogeissus leiocarpus*, *Kaya senegalensis*, *Euphorbia hirta*, *Annona senegalensis* and *Parquetina homalium* on adult males of *O. ochengi* and *Caenorhabditis elegans* concluding that these plants possess bioactive elements with anthelmintic properties. The other possible anthelmintic effect of tannins is that they can bind to glycoproteins on the cuticle of the parasite and can lead to its death (Djafsia *et al.*, 2018). This demonstrates the possible modes of action of our extracts because the chemical families are mainly tannins.

The LC₅₀ values obtained in 72 hours (0.05 mg/mL for roots and leaves, 0.06 mg/mL for fruits and bark) are in line with those of Enock *et al.*, (2022) who demonstrated that the medicinal properties of *Indigofera tinctoria* are found in the bark, leaves and roots to treat several diseases.

The test on the microfilariae of the part which has the most effect was carried out. After 24 hours, we obtain 100% mortality at a concentration of 0.1 mg/mL. These results are in line with those of Cho-Ngwa (2010) on the microfilariae of *O. ochengi* with two local plants (*Homalium africanum* and *Margueritaria discoidea*).

Mortality caused by ivermectin is 100% at a concentration of 0.5 mg/ml in 48 hours and 72 hours. These results contradict those of Elodie *et al.*, (2020) which attest that the in vivo administration of ivermectin would have low lethal activity on *O. volvulus*. On the other hand, the administration of higher doses can increase side effects in vivo but also act more markedly by stopping the production of microfilariae by adults causing moderate adult mortality (Djafsia *et al.*, 2018). All this work confirms that the present study will contribute to strengthening existing knowledge as well as the use of these medicinal plants as anthelmintics and especially *Vitellaria paradoxa*.

CONCLUSION

In short, our research consists of evaluating the antifilarial properties of a local plant (*V. paradoxa*.) on the parasitic nematode *O. ochengi* at different concentrations (0.1; 0.2; 0.3; 0.4 mg/ mL). The results obtained made it possible to affirm that the extracts of the roots, fruits, leaves and bark of *V. paradoxa* have antifilarial activity on adult worms of *O. ochengi*. After 72 hours of incubation at 37°C, the mortality of *O. ochengi* worms reached 100% at a concentration of 0.1 mg/ml for roots and leaves. The hydroethanolic and aqueous extracts of the trunk barks of *V. paradoxa* could be a potential source of natural biomolecules to fight against parasitic infections in Chad and elsewhere.

Conflicts of Interest: For this article, the authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

MAD developed and ensured the collection of the sample in the field, carried out the manipulation in the laboratory as well as the writing of the first draft. NB directed the manipulation in the laboratory and contributed to the correction and scientific orientation of the writing. MM coordinated the entire work.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Research, Diagnostic and Scientific Expertise Laboratory, the Toxicology and Pharmacology Unit of the Faculty of Human Health Sciences of N'Djamena/Chad for providing the necessary materials for carrying out this study.

REFERENCES

- Amina, M., Younoussa, L., Fanta, Y. S. A., Nguezeze, Y., Okah-Nnane, N. H., Bitja-Nyom, A. R., & Ndjonka, D. (2023). *In vitro* nematocidal potential of hydro-ethanolic and aqueous extracts of *Calotropis procera* (Aiton) W.T. Aiton, 1811 (Apocynaceae) and *Faidherbia albida* (Delile) A. Chev., 1934 (Fabaceae) against *Onchocerca ochengi* and *Caenorhabditis elegans*. *Heliyon*, 9 (2023), 1-14.
- Aránzazu, G., Ana, M., Sahagún, P., José, D., Névida, F., Matilde S., & Juan, J. (2012). The

- Pharmacokinetics and Interactions of Ivermectin in Humans. *Infection and Immunity*, 69, 4313-4319.
- Bakarnga, V. I., Potaïso, D., Bessimbaye, N., Issa, R. A., Brahim, B. O., Abdoullahi, H. O., Mbaïgolem, B. V., & Abdelsalam, T. (2022). Anti-radical and antibacterial activities of the ethanolic extract bark of *Anogeissus leiocarpus* (Guill. Et Perr) from Chad. *Scholars Academic Journal of Biosciences*, 10(11), 127-132.
 - Bayala, J., Sanon, Z., Bazié, P., Sanou, J., Rroupsard, O., Jourdan, C., ... & Yidana, J. (2018). Relationships between climate at origin and seedling traits in eight Panafrican provenances of *Vitellaria paradoxa* CF Gaertn. under imposed drought stress. *Agroforestry systems*, 92, 1455-1467.
 - Bouraïma Mama Sambo. (2021). Medicinal plants used in the treatment of pathologies of small ruminants in the Borgou department: case of the Communes of Kalalé, Pèrèrè, and Nikki. End of training thesis defended at the University of Abomey-Calavi, 44p.
 - Cho-Ngwa, F., Abongwa, M., Moses, N., & Kennedy, D. (2010). Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca ochengi*. *BMC Complementary and Alternative Medicine*, 10(62), 1-7.
 - Dikti, V. J., Kalmobé, J., Djafsia, B., Schmidt, T. J., Liébau, E., & Ndjonka, D. (2017). Activité anti-*onchocerca* et anti-*Caenorhabditis* d'un extrait hydro-alcoolique des fruits d'*Acacia nilotica* et de certains dérivés de proanthocyanidines. *Molecules*, 22(5), 1-19.
 - Djafsia, B., Dieudonné, N., Albert, E., Kingsley, M., Archille, P., Nancy, N. N., Jacqueline, D. V., Babette, A., Ralf, K., Silke, V. H., Alfons, R., Mbunkah, D. A., Eva L., & Norbert, W. B. (2018). *Onchocerca* - infected cattle produce strong antibody responses to excretory secretory proteins released from adult male *Onchocerca ochengi* worms. *BMC Infectious Diseases*, 18(200), 1-10.
 - Eisenbarth, A., Mbunkah, D. A., & Renz, A. (2016). Ongoing Transmission of *Onchocerca volvulus* after 25 Years of Annual Ivermectin Mass Treatments in the Vina du Nord River Valley, in North Cameroon. *PLOS Neglected Tropical Diseases*, 1-16. DOI: 10.1371/journal.pntd.0004392.
 - Elodie, M. M., Noël, J. N., Ngwafu, N. N., Adeline, S. Y. F., Francis, N., Siméon, F. K., & Dieudonné, N. (2020). *In vitro* Anthelmintic Activities of Extracts and Fractions of *Cosmos sulphureus* Cav, Against *Onchocerca ochengi*. *Journal of Diseases and Medicinal Plants*, 6(1), 22-30.
 - Enock, E. R., Benoît, B. K., Bertrand, N. M., Mathieu, D. Francis, N., & Dieudonné, N. (2022). Antifilarial Activity of the Methanolic Extract of *Indigofera tinctoria* (Fabaceae) on Bovine Parasites (*Onchocerca ochengi*). *Journal of Parasitology Research*, 2022(7828551), 1-10.
 - Kalmobé, J., Dieudonné, N., Djafsia, B., Jacqueline, D. V., & Eva, L. (2017). Phytochemical analysis and *in vitro* anthelmintic activity of *Lophira lanceolata* (Ochnaceae) on the bovine parasite *Onchocerca ochengi* and on drug resistant strains of the free-living nematode *Caenorhabditis elegans*. *BMC Complementary and Alternative Medicine*, 17(404), 1-12.
 - Kalmobé, J., Jacqueline, D. V., Djafsia, B., Honoré Ndouwé, T. M., Simeon, K. F., & Dieudonné, N. (2023). *In vitro* anthelmintic activity and secondary metabolites analysis of leaves of *Aloe vera* (L.) Burm. f. (Xanthorrhoeaceae) against bovine parasite *Onchocerca ochengi* and drug resistant strains of the free-living nematode *Caenorhabditis elegans*. *Research Square*, (Suppl), 1-27. DOI: <https://doi.org/10.21203/rs.3.rs-3086594/v1>
 - Narhari, D., Durajan, G., Sharif, H., Sheikh, Z. R., & Nirmal, K. S. (2015). Phytochemical screening and *in vitro* anthelmintic activity of methanol extract of *Terminalia citrina* leaves. *Asian Pacific Journal of Tropical Disease*, 5(Suppl 1), S166-S168.
 - Ndjonka, D., Ajonina-Ekoti, I., Djafsia, B., Lüersen, K., Abladam, E., & Liebau, E. (2012). *Anogeissus leiocarpus* extract on the parasite nematode *Onchocerca ochengi* and on drug resistant mutant strains of the free-living nematode *Caenorhabditis elegans*. *Veterinary Parasitology*, 190(1-2), 136-142.
 - Ndjonka, D., Ayoub, M., Ahamat, A., Djafsia, B., & Ndouwe, T. M. H. (2017). *In vivo* Toxicity Study and Antifilarial Activity of Four Plants from Nord-Cameroon. *European Journal of Medicinal Plants*, 19(3), 1-12.
 - Ndjonka, D., Djafsia, B., & Liebau, E. (2018). Review on medicinal plants and natural compounds as anti-*Onchocerca* agent's nematodes. *Parasitology research*, 117, 2697-2713.
 - WHO. (2022). WHO Expert Committee on Onchocerciasis third series of technical reports. 32, 75-80.
 - WHO. (2011). *Onchocerca ochengi*: epidemiological evidence of cross-protection against *Onchocerca volvulus* in man. 97p.
 - WHO. (2013). Compte-rendu de la mission d'évaluation épidémiologiques phase 1a et 1b au Tchad du 4 Juillet au 13 Aout 2013. 15p.