Heliyon 9 (2023) e16379

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

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 (Fabacae) against *Onchocerca ochengi* and *Caenorhabditis elegans*

Amina Mamat [a,b], Younoussa Lame [d,*], Fanta Yadang Sabine Adeline [a,b], Nguezeye Yvette [a,b], Okah-Nnane Ndode Herman [c], Bitja-Nyom Arnold Roger [a], Ndjonka Dieudonné [a]

[a] *Department of Biological Sciences, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon*
[b] *Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plant Studies, P.O. Box 13033, Yaoundé, Cameroon*
[c] *Institute of Agricultural Research for Development (IRAD), Veterinary Research Laboratory, Wakwa Regional Center, P.O. Box 65, Ngaoundéré, Cameroon*
[d] *Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1 P.O. Box 812, Yaoundé, Cameroon*

ARTICLE INFO

Keywords:
Medicinal plants
Plant extracts
Secondary metabolites
Blindness
Onchocerca ochengi
Caenorhabditis elegans

ABSTRACT

Onchocerciasis caused by *Onchocerca volvulus* Leuckart, 1893 is the second-world infection responsible for human blindness. Except Ivermectin which has as targets the microfilariae of that parasite, specific treatment for this disease does not exist and in developing countries, medicinal plants seem to remedy that health problem. For that, aqueous and hydro-ethanolic leaf, bark, and root extracts of *Calotropis procera* and *Faidherbia albida* were evaluated *in vitro*, against the most popular bovine model, *Onchocerca ochengi* and the free-resistant nematode *Caenorhabditis elegans*. *O. ochengi* microfilariae and adults extracted from the bovine nodules and skins as well as the free strains of *C. elegans* were exposed to the various concentrations of the plant parts extracts and Ivermectin. In results, all the plant parts extracts were rich in tannins, saponins, alkaloids, flavonoids, phenols, coumarins, and glycosides. Phenols (175.45 ± 0.01 mg EGA/g DM), flavonoids (158.98 ± 0.05 mg EC/g DM), and tannins (89.98 ± 2.56 mg ETA/g DM) contents were high in the bark hydro-ethanolic extract of *F. albida*. The leaf hydro-ethanolic extract of *F. albida* induced high activity against *O. ochengi* microfilariae (CL_{50} = 0.13 mg/mL). The bark hydro-ethanolic extract of *F. albida* was also the most effective on *O. ochengi* adults and particularly on female adults (CL_{50} = 0.18 mg/mL). Against the parasite strain resistant to Ivermectin, *F. albida* leaf hydro-ethanolic extract appeared more active with CL_{50} = 0.13 mg/mL. Similarly, the bark hydro-ethanolic extract of *F. albida* was the most potent on the wild strain of *C. elegans*. Thus, this study validates the use of these plants by traditional healers in the management of onchocerciasis and suggests a new source of isolation of the potential plant compounds against *Onchocerca.*

* Corresponding author.
E-mail address: younoussalame@yahoo.com (Y. Lame).

https://doi.org/10.1016/j.heliyon.2023.e16379
Received 29 January 2023; Received in revised form 27 April 2023; Accepted 15 May 2023
Available online 18 May 2023

1. Introduction

Among the neglected tropical diseases, lymphatic filariasis and onchocerciasis constitute the principal public health problem causing deformities to the populations of Sub-Saharan Africa [1]. Also called river blindness, onchocerciasis is a filarial infection caused by *Onchocerca volvulus* and transmitted to humans through the bites of a hematophagous blackfly belonging to the genera of *Similium* [2]. According to The Global Burden of Disease Study report, an estimated 220 million people require preventive anti-onchocerciasis chemotherapy in 2017, of which 14.6 million of the infected persons already had a skin infection and 1.15 million were blind [3]. The infected people live mostly in sub-Saharan Africa (in 31 African countries) representing 99% of the worldwide infected people [4]. That disease represents the second cause of infectious blindness. The rural communities living close to the agitated rivers are the most exposed because of the high reproductive rate of black flies, the main vector of that disease [5]. That pathology appears through cutaneous attacks, invalidity, and ocular disorders leading to blindness, depriving the patients of their work capacity [6]. In fact, the adults of *O. volvulus* live at least 15 years in the subcutaneous nodules and can produce a million microfilariae. Then, these microfilariae parasitize the skin and eye tissues and have serious consequences like disfiguring dermatitis, muscular atrophy, intense and unbearable itching as well as visual weakening and blindness [7]. Deprived of their most productive persons, villages with fertile and rich valleys are the first to suffer from the socio-economic impacts and are forced to migrate to the less-infested zones [8].

To face onchocerciasis, the control methods based on the treatment by eliminating the worms in the patients and disease prevention by the reduction of the vector bites are employed [9]. As the anti-vector control was given up, chemotherapy remains the main control method to combat onchocerciasis. However, Ivermectin which is the only drug recommended in the treatment of that disease has as a target only the microfilariae. Unfortunately, the adults of *O. volvulus* who can live up to 18 years in the human being cause real damage and handicap to the patients [10].

In previous studies, preparations from medicinal plants were identified as alternative remedies in the treatment of several diseases [11]. Thus, the filaricidal activities of certain medicinal plants were reported [12–17]. Some phytochemical constituents like polyphenols, tannins and isolated compounds including voacamine, polycarpol, voacangine, ellagic acid, gentisic acid, gallic acid, (−)-epigallocatechin 3-*O*-gallate, 3-*O*-acetyl aleuritolic acid, (+)-catechin-3-*O*-gallate, (−)-epicatechin-3-*O*-gallate, (+)-gallocatechin, (−)-epigallocatechin, and (−)-epigallocatechin-3-*O*-gallate were reported to possess strong activity against onchocerca [14,17].

Calotropis procera is a small tree reaching 2.5–6 m in height largely distributed in Asia and subtropical Africa. The anthelmintic effects of the different parts of *Calotropis procera* on *Haemonchus contortus* were also reported [18]. *C. procera* extracts were revealed to possess antibacterial, analgesic and anti-nociceptive activity [19], wound healing and antioxidant activity [20], antidiabetic effect [21], myocardial infarction and schizontocidal activity [22], anticancer [23], antimicrobial, antiulcer, antifertility, antidiarrheal, estrogenic functionality and anticonvulsant effects [24], anti-inflammatory activity [25], larvicidal activity [26], dermatophytic activity [27] and immunomodulatory property [28–30].

Faidherbia albida is a tree 20–30 m in height and 1–1.5 m in diameter. It has been reported the different parts of the plant possess antioxidant and antimicrobial activities [31], antibacterial properties [32], antimalarial effect [33], antidiabetic and anti-hyperlipidemic activities [34].

Although these 2 plant species are used locally by the traditional healers of the North and East regions of Cameroon to treat onchocerciasis, but from our knowledge, the anti-Onchocerca and anti-Ceanorhabditis properties of the 2 plants have not been yet documented. Because of the similarities and the close relative of the bovine parasite *Onchocerca ochengi* with the human parasite *O. volvulus*, the bovine parasite remains the suitable model of the anti-onchocercal assessment since they are cheaper and easily to obtain in Africa. In fact, the two parasites (*O. volvulus and O. ochengi*) microfilariae are both sensitive to the commercial drug ivermectin and they share the same vector (black fly) [35]. Moreover, both female and male adults of the two nematods species are found in intradermal or subcutaneous of their hosts (human and bovine). Trees [36] demonstrated the effect of ivermectin on the bovine parasite affect also *O. volvulus*.

To overcome the problem of resistance, both wild and ivermectin-resistant strains of Caenorhabditis *elegans* where used in this study. In fact, the free-living soil nematode *C. elegans* is a simplest nematod model largely used in anthelmintic drug screening. This because the parasite is easy and cheaper to grow and maintain, it has a short life cycle with numerous progeny [37]. However, resistance to ivermectin in nematodes are increasingly reported and that high-level resistance to the antiparasitic drug ivermectin implied a simultaneous mutation of three genes, avr-14, avr-15, and glc-1, encoding glutamate-gated chloride channel (GluCl) α-type subunits [38].

The objective of this present work aimed to identify some phytochemical principles in the aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of *F. albida* and *C. procera* and to evaluate their nematocidal properties *in vitro* on the parasitic stages of *O. ochengi* and on the free nematode strains (wild and resistant to the ivermectin) of *C. elegans*.

2. Materials and methods

2.1. Collection and processing of the plant materials

Samples of the different parts (leaves, barks, and roots) of *C. procera* were collected from Touboro in the North region in July 2020 while those of *F. albida* were harvested in October 2020 from Mora in the Far-north region of Cameroon. These plants were identified at the National Herbarium of Cameroon at Yaounde under the registration number 45506/HNC for *F. albida* and 4751/SRFC for *C. procera* where their vouchers were deposited. The parts of each plant species were cleaned with tap water, cut into small pieces, and then

shade-dried for 4 weeks. The dried parts of each plant were separately crushed in the wood mortar and passed through a 0.4 mm mesh size sieve. Each plant part powder obtained was kept in the dark glass until their use for extraction.

2.2. Hydro-ethanolic and aqueous extractions of the plant parts

The hydro-ethanolic extracts were obtained by macerating each plant powder in a mixture of ethanol and distilled water (70:30 V/V) in a ratio of 100 g powder for 1000 mL of hydro-ethanolic solvent under reduced agitation during 48 h at the ambient temperature. Then, each macerate was centrifuged at 3500 rpm for 10 min. The recovered supernatant from each macerate was filtered using Whatman no.1 filter paper. Each filtrate obtained was concentrated using a rotavapor. Each tick extract obtained was placed for 72 h in a ventilated drying oven set at 30 °C for complete drying.

To obtain the aqueous extract of each part of *C. procera* and *F. albida*, each plant powder (100 g) was macerated in 1000 mL of distilled water and boiled for 10 min. After cooling, each macerate was filtered using Whatman no.1 filter paper. Each filtrate obtained was dried in the oven set at 40 °C for 72 h.

At last, the dry aqueous and hydro-ethanolic extracts of each part of *C. procera* and *F. albida* obtained were weighed and each extract yield was calculated according to the following formula [39].

$$\text{Extraction Yield}(\%) = \frac{\text{Weight of the extract obtained (g)}}{\text{Weight of the plant powder used (g)}} \times 100$$

2.3. Qualitative phytochemical screening

The aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of *C. procera* and *F. albida* were screened to identify some phytochemical compounds such as tannins, polyphenols, saponins, flavonoids, alkaloids, anthocyanins, anthracenes, terpenoids, coumarins, and glycosides according to the methods of Sofowora [40] and Harbone [41].

2.4. Dosage of some phytochemical constituents

From the results of the qualitative phytochemical screening, flavonoids, tannins, and phenols were furthermore submitted to quantitative screening to determine the quantity of these compounds in the plant parts extracts following the method of [42].

2.5. Sources of the nematodes species used

2.5.1. Isolation and culture of O. ochengi male and female adults

The method described by Ndjonka et al. [43] was followed to isolate the adults (males and females) of *O. ochengi*. Fresh pieces of umbilical bovine skin containing enough quantity of palpable nodules were collected from Ngaoundere township slaughter-house and were transported to the laboratory of parasitology of IRAD (Institute of Agricultural Research for Development) at Wakwa in Ngaoundere, Cameroon. The skins were carefully washed successively with tap water and distilled water, then drained and completely covered with 70% ethanol which could evaporate itself under a hood with laminar flow in a sterile environment. The nodules of these cleaned skins were extirpated using a lancet, then put directly in PBS solution before the dissection. The dissection of the nodules was done with an assembled scalpel, a thick grip, a fine grip, and an assembled needle. The dissection of the nodules released the pale orange-yellow masses containing primarily viable males and females of *O. ochengi*. The separation of the males from the females was done using a fine grip and assembled needle under the microscope. The males with more mobility than the females were collected with a fine grip and transferred into a sterile PBS solution. The same operation was done for the females.

2.5.2. Extraction and culture of O. ochengi microfilariae

O. ochengi microfilariae were extracted from the infected bovine skins provided by the township slaughterhouse of Ngaoundere. The skin was firmly attached to a piece of cylindrical autoclaved wood and approximately 0.5–1 mm intersected cuts were made in the dermic layers of the bovine skin. Each biopsy portion was transferred into a sterilized cylinder glass and a suitable volume of complete culture medium (CCM) was added to just cover the whole skin and then incubated for 4–6 h at ambient temperature. The very mobile microfilariae that emerged were concentrated by centrifugation (400 rpm for 10 min) and were quantified under the microscope.

2.5.3. Multiplication, synchronization, and culture of Caenorhabditis elegans

Strains of *C. elegans* (wild strain and mutant strain resistant to Ivermectin VC722) collected from the *Caenorhabditis elegans* Genetic Center of Minneapolis/USA were multiplied in Nematode Growth Medium (NGM-Agar) according to the method described by Ndjonka et al. [43]. In Petri dishes containing a solidified NGM-Agar, 600 µL of *Escherichia coli* OP-50 were added and then dried for 1 h in the ventilated oven. After drying, each *C. elegans* strain was transferred onto the medium culture using a scalpel. The petri dishes were incubated at 18 °C during 48 h in CO_2 incubator.

To synchronize *Caenorhabditis elegans*, the worm multiplication was assessed following the methods described by Ndjonka et al. [43] and Smith et al. [44]. In the medium containing gravid adult worms and eggs, distilled water (3–5 mL) was added until a complete immersion of the medium surface and kept for 5–10 min. The supernatant was thereafter recovered in 5 mL Eppendorf tubes, then centrifuged at 8000 rpm for 1 min using a cooled centrifugal machine (4 °C). The residue (containing several adults and eggs) was

Table 1
Extraction yields and physical characteristics of the extracts.

Plant species	Extracts	Plant parts	Colour	Aspect	Yield (%)
Faidherbia albida	Aqueous	Leaves	Green	Sticky- Sticking	18.27
		Barks	Black	Crystal	19.8
		Roots	Grey	Crystal	14.61
	Hydro-ethanolic	Leaves	Green	Crystal	10.59
		Barks	Brown	Crystal	9.85
		Roots	Grey	Crystal	15.71
Calotropis procera	Aqueous	Leaves	Green	Sticky- Sticking	10.11
		Barks	Brown	Crystal	7.75
		Roots	Brown	Crystal	10.10
	Hydro-ethanolic	Leaves	Green	Sticky-Tender	20.45
		Barks	Brown	Sticky-Tender	19.81
		Roots	Black	Crystal	19.9

recovered and then, disinfected for 6 min in a solution of Chlorox. The mixture residue + chlorox was once more centrifuged at 8000 rpm for 1 min. The supernatant was discarded and the residue containing eggs was rinsed 3 times (by centrifuging at 8000 rpm for 1 min at each rinsing) in 3 mL buffer solution (M9-buffer). The residue was kept in 1 mL M9-buffer in the incubator set at 18 °C for 24 h, then dispatched in petri dishes (at 100 μL per Petri dish) containing NGM (Nematode Growth Medium) sown with *E. coli* OP-50. After 24–30 h of incubation, the eggs hatched into larvae, and these having the same age were transferred into a new Petri dish for the pharmacological tests.

2.6. Nematocidal tests of the plant part extracts

After isolation of the males and females of *O. ochengi*, the worms were immersed in complete culture medium (MCC) (Rpmi-1640 added/mL with penicillin, 200 μg/mL of streptomycin and 2.5 μg/mL of amphotericin B, pH = 7.4) in 96 wells plates. The cultures were incubated at 37 °C under CO_2 5% atmosphere in humidified air in an incubator Heracell-150 CO_2 for 2 days after the addition of the plant extracts or Ivermectin previously dissolved in distilled water at 0.125, 0.250, 0.375, 0.500, 0.625, 0.750 and 0.875 mg/mL; concentrations maintained after the preliminary screening tests.

For *the O. ochengi* microfilariae test, 100 pis of CCM containing 15–20 microfilariae were transferred in each well of a microtitration culture plate of 96 wells. After the addition of the same concentrations (0.125, 0.250, 0.375, 0.500, 0.625, 0.750 and 0.875 mg/mL) of the plant extracts or Ivermectin dissolved in distilled water, microfilariae test plates were incubated at 37 °C under CO_2 5% atmosphere in humidified air incubator (Heracell-150 CO_2, Thermo Electron, Germany) during 48 h.

After the synchronization of *C. elegans*, the worms were transferred into petri dishes (15 worms/well) containing medium culture NGM-Agar (3 mL). In each Petri dish containing worms, concentrations of 0.125, 0.250, 0.375, 0.500, 0.625, 0.750 and 0.875 mg/mL of the plant extracts or Ivermectin were added and incubated at 18 °C under 5% CO_2 atmosphere. Inhibiting effects were recorded in terms of worm death after 48 h [43].

2.7. Revelation test of worms motility and mortality

The viability of the worms was determined by microscopic examination using an inverted microscope (euro mix, Holland) and by colorimetry using resazurin [43]. Indeed, microfilariae in culture during 48 h were revealed by microscopy in which inhibition was complete when no movement was observable visually.

Using the colorimetric qualitative test of revelation to resazurin or Alamar Blue as described by Chen et al. [45], the worms incubated at 37 °C for 48 h were removed and brought under the disinfected hood. Worms were removed from their culture, washed in PBS solution, and introduced into multiwell plates containing each, 500 μL of RPMI solution follow by adding resazurin (5 μL, concentration 0.5 mg/mL) and then incubated in a CO_2 incubator at 37 °C for 30 min. After incubation, the worms were observed under the microscope, and the colors of the worms determined whether they are alive (pink colour) or dead (blue colour) [46].

2.8. Statistical analyses

Data from the mortality of the nematodes were submitted to the analysis of variance using SPSS 16.0 software. For the comparison of the means, the Tukey test (P = 0.05) was employed. Probit analysis [47] was used to determine the lethal concentrations (LC_{50} and LC_{90}) of the plant parts extracts that caused 50% and 95% of microfilariae or adults of the nematodes assessed.

3. Results

3.1. Yields and physical characteristics of the extracts

The extraction yields varied from 7.75 to 19.8% for the aqueous extracts and from 9.85 to 20.45% for the hydro-ethanolic extracts

Table 2
Phytochemical composition of leaf, bark, and root extracts of *C. procera* and *F. albida*.

Phytochemical Compounds	*F. albida* extracts						*C. procera* extracts					
	Aqueous			Hydro-ethanolic			Aqueous			Hydro-ethanolic		
	Lv	Bk	Rt	Lv	Bk	Rt	Lv	Bk	Rt	Lv	Bk	Rt
Tannins	+	+	+	+	+	+	+	+	+	+		
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloïds	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	–	–	–	–	–	+	–	+	+	–	+	+
Anthocyanins	–	–	–	–	–	–	+	+	+	+	+	+
Sterols/Terpenes	+	+	+	+	+	–	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+

Lv = Leaves, Bk= Barks and Rt = Roots; - = absent and + = present.

Table 3
Quantity of phenols, flavonoids, and tannins in the *F. albida* and *C. procera* extracts.

Plant species	Extracts	Plant parts	Phenols (mg EGA/g DM)	Flavonoids (mg EC/g DM)	Tannins (mg ETA/g DM)
F. albida	Aqueous	Leaves	74.20 ± 1.21	61.57 ± 2.59	48.21 ± 23.45
		Barks	92.06 ± 0.96	83.24 ± 1.32	31.40 ± 12.14
		Roots	87.24 ± 0.66	71.02 ± 0.21	27.59 ± 21.11
	Hydro-ethanolic	Leaves	146.65 ± 0.02	125.11 ± 0.02	66.75 ± 23.31
		Barks	175.45 ± 0.01	158.98 ± 0.05	89.98 ± 2.56
		Roots	145.62 ± 0.01	104.3 ± 0.01	75.34 ± 0.00
C. procera	Aqueous	Leaves	88.51 ± 0.01	45.39 ± 0.00	57.10 ± 0.20
		Barks	81.18 ± 0.01	68.89 ± 0.11	49.12 ± 0.12
		Roots	77.47 ± 0.04	75.11 ± 0.14	47.19 ± 0.00
	Hydro-ethanolic	Leaves	132.44 ± 0.05	149.99 ± 0.02	65.82 ± 0.00
		Barks	99.73 ± 0.03	104.65 ± 0.10	51.13 ± 0.04
		Roots	141.31 ± 0.04	99.35 ± 0.23	54.89 ± 0.55

mg EGA/g DM = milligram equivalent of gallic acid/gram of dry matter; mg EC/g DM = milligram equivalent of catechin/gram of dry matter; mg ETA/g DM = milligram equivalent of tannic acid/gram of dry matter.

of the two plant parts (Table 1). The highest yield was obtained with the hydro-ethanolic solvent extraction of the leaves of *C. procera* (29.51%) while the aqueous extraction of the barks of *C. procera* presented the poorest yield of 7.75%. Concerning the physical characteristics of the plant extracts, their colors grey, brown, black, and green were recorded, while their aspects varied from crystals to sticky-tender pastes.

3.2. Phytochemical screening of leaf, bark, and root extracts of C. procera and F. albida

Results of the phytochemical screening of the aqueous and hydro-ethanolic extracts of the different parts of *F. albida* and *C. procera* are presented in Table 2. In *F. albida* extracts, tannins, phenols, saponins, flavonoids, alkaloids, coumarins, and glycosides are present in both aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of the plant. Sterols and terpenoids were also present in the aqueous of the 3 plant parts and hydro-ethanolic extracts of the leaves and barks of the plant species. In both aqueous and hydro-ethanolic extracts of *C. procera,* tannins, phenols, saponins, flavonoids, alkaloids, sterols, terpenoids, coumarins, and glycosides were present. Anthraquinones were also present in the aqueous and hydro-ethanolic barks and roots of the plant species.

3.3. Quantitative screening of some contents of the plant extracts

Table 3 presents the phenols, flavonoids, and tannins quantities in the leaf, bark, and root extracts of *F. albida* and *C. procera*. Globally, the phytochemical contents are high in the hydro-ethanolic extracts of *F. albida* and *C. procera* compared to the aqueous extracts of the two plants. Phenols (175.45 ± 0.01 mg EGA/g DM), flavonoids (158.98 ± 0.05 mg EC/g DM), and tannins (89.98 ± 2.56 mg ETA/g DM) were highly found in the bark hydro-ethanolic extract of *F. albida*.

3.4. Nematocidal activities

3.4.1. Effect of Faidherbia albida parts extracts and Ivermectin against O. ochengi

Fig. 1 presents the nematocidal activity of the hydro-ethanolic (Fig. A–C) and aqueous (Fig. 1D–F) extracts of the leaves, barks, and roots of *F. albida* and Ivermectin against males (Fig. 1A and D), females (Fig. 1B and E), and microfilariae (Fig. 1C and F) of *O. ochengi*.

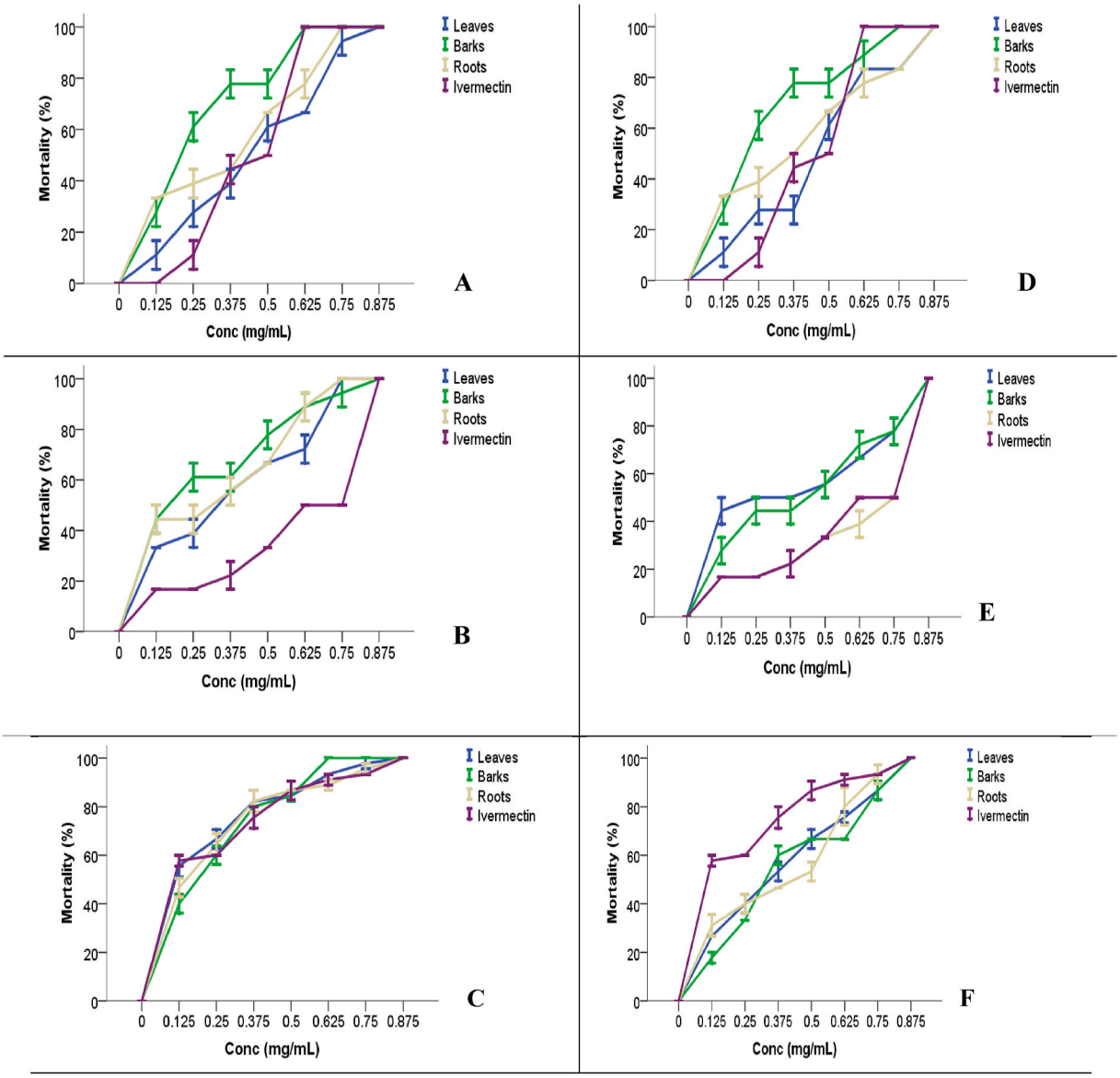

Fig. 1. Mortality of *Onchocerca ochengi* adults males (A and D), females (B and E), and microfilariae (C and F) treated with hydro-ethanolic extracts (A, B, and C) and aqueous extracts (D, E, and F) of the leaves, barks, and roots of *Faidherbia albida.* In each graph, concentration 0 mg/mL represents the negative control in which no plant extract is added.

In general, these extracts induced significant mortality concentration-dependent against the sensitive strain of *O. ochengi* which increased significantly with the increased concentrations of the extracts of the various parts of the plant as well as the reference drug.

Regarding LC_{50} values (Table 4), the males of *O. ochengi* were more sensitive to the barks hydro-ethanolic extract (LC_{50} = 0.20 mg/mL) of *F. albida* compared to roots (LC_{50} = 0.27 mg/mL) and leaves (LC_{50} = 0.27 mg/mL) extracts as well as Ivermectin (LC_{50} = 0.40 mg/mL). Similarly, the hydro-ethanolic extracts of the barks (LC_{50} = 0.18 mg/mL) and the roots (LC_{50} = 0.21 mg/mL) were more effective on the females of *O. ochengi*, compared to the leaves extract (LC_{50} = 0.27 mg/mL) of the plant and Ivermectin (LC_{50} = 0.55 mg/mL). Against microfilariae, Ivermectine (LC_{50} = 0.13 mg/mL) and hydro-ethanolic extract of the leaves were more toxic compared to the hydro-ethanolic extracts of the roots (LC_{50} = 0.15 mg/mL), and the barks (LC_{50} = 0.17 mg/mL) of the plant.

Concerning the aqueous extracts of *F. albida* (Table 4), the males of *O. ochengi* were more sensitive to the barks aqueous extract (LC_{50} = 0.20 mg/mL) compared to the roots aqueous extract (LC_{50} = 0.27 mg/mL) and leaf extract (LC_{50} = 0.37 mg/mL) as well as Ivermectin (LC_{50} = 0.40 mg/mL). On the females of *O. ochengi*, the leaf aqueous extract (LC_{50} = 0.23 mg/mL) appeared as most toxic compared to the barks (LC_{50} = 0.30 mg/mL) and roots extracts (LC_{50} = 0.58 mg/mL) as well as Ivermectin (LC_{50} = 0.55 mg/mL). Against the microfilariae of *O. ochengi*, Ivermectine (LC_{50} = 0.13 mg/mL) was the most toxic compared to the aqueous extracts of the leaves (LC_{50} = 0.28 mg/mL), barks (LC_{50} = 0.31 mg/mL) and roots (LC_{50} = 0.28 mg/mL) of *F. albida.*

Table 4
LC_{50} and LC_{95} (mg/mL) of hydro-ethanolic and aqueous extracts of the leaves, barks, and roots of *F. albida* against males, females, and microfilariae of *O. ochengi*.

Extracts	Parasite Stages	Plant parts	Slope±SE	R^2	LC_{50} (CI 95%)	LC_{95} (CI 95%)	χ^2
Hydro-ethanolic	Males	Leaves	3.46 ± 0.13	0.93	0.37 (0.31–042)	1.10 (0.87–1.63)	199.10***
		Barks	3.33 ± 0.14	0.79	0.20 (0.16–0.24)	0.63 (0.52–0.84)	139.05***
		Roots	2.60 ± 0.11	0.92	0.27 (0.20–0.33)	1.16 (0.84–2.07)	217.50***
	Females	Leaves	2.51 ± 0.11	0.92	0.26 (0.20–0.32)	1.18 (0.86–2.07)	190.29***
		Barks	2.19 ± 0.17	0.84	0.18 (0.12–0.23)	1.02 (0.74–1.78)	154.74***
		Roots	2.37 ± 0.11	0.88	0.21 (0.14–0.27)	1.07 (0.76–2.09)	226.59***
	Microfilariae	Leaves	2.18 ± 0.12	0.86	0.13 (0.09–0.16)	0.73 (0.60–0.99)	66.76***
		Barks	3.06 ± 0.13	0.85	0.17 (0.14–0.20)	0.61 (0.52–0.76)	80.55***
		Roots	2.31 ± 0.12	0.83	0.15 (0.12–0.17)	0.77 (0.65–0.98)	51.28***
Aqueous	Males	Leaves	3.51 ± 0.13	0.90	0.37 (0.32–0.43)	1.11 (0.88–1.62)	197.64***
		Barks	2.97 ± 0.13	0.79	0.20 (0.16–0.24)	0.74 (0.60–0.99)	125.08***
		Roots	2.24 ± 0.11	0.95	0.27 (0.22–0.32)	1.47 (1.08–2.42)	114.49***
	Females	Leaves	1.49 ± 0.10	0.80	0.23 (0.14–0.31)	3.00 (1.56–14.92)	164.78***
		Barks	2.13 ± 0.11	0.86	0.30 (0.23–0.37)	1.81 (1.21–3.90)	167.67***
		Roots	2.26 ± 0.12	0.75	0.58 (0.45–0.83)	3.11 (1.68–14.33)	292.64***
	Microfilariae	Leaves	2.49 ± 0.11	0.96	0.28 (0.24–0.32)	1.30 (1.02–1.87)	93.07***
		Barks	2.79 ± 0.12	0.93	0.31 (0.27–0.36)	1.23 (0.99–1.68)	93.10***
		Roots	2.45 ± 0.11	0.90	0.28 (0.21–0.35)	1.35 (0.94–2.61)	213.69***
Ivermectin	Males	–	7.14 ± 0.28	0.90	0.40 (0.36–0.44)	0.68 (0.61–0.82)	182.28***
	Females	–	2.36 ± 0.12	0.79	0.55 (0.44–0.74)	2.74 (1.59–9.50)	267.09***
	Microfilariae	–	1.97 ± 0.12	0.89	0.13 (0.09–0.16)	0.90 (0.71–1.32)	71.73***

SE= Standard Error; R^2 = Coefficient of determination; LC = Lethal concentration; CI= Confident interval; χ^2 = Chi-square; ***P < 0.001.

3.4.2. *Effect of Calotropis procera parts extracts and Ivermectin against O. ochengi*

The nematocidal activity of the hydro-ethanolic (Fig. 2A–C) and aqueous (Fig. 2D–F) extracts of the leaves, barks, and roots of *C. procera* against males (Fig. 2A and D), females (Fig. 2B and E), and microfilariae (Fig. 2C and F) of *O. ochengi* is presented in Fig. 2. Globally, all the extracts of *C. procera* and the reference drug caused significant concentration-dependent mortality and that activity increased with increasing concentrations of the plant extracts and also for the reference drug.

From the LC_{50} (mg/mL) values obtained (Table 5), the bark hydro-ethanolic extract (LC_{50} = 0.24 mg/mL) of the plant appeared to be more toxic on the males of *O. ochengi* compared to the leaves (LC_{50} = 0.30 mg/mL) and roots (LC_{50} = 0.44 mg/mL) extracts of the plant as well as Ivermectin (LC_{50} = 0.40 mg/mL). On the females, it was rather the leaf hydro-ethanolic extract (LC_{50} = 0.18 mg/mL) of the plant which was more effective compared to the barks (LC_{50} = 0.29 mg/mL) and roots (LC_{50} = 0.30 mg/mL) extracts of the plant as well as Ivermectin (LC_{50} = 0.55 mg/mL). On the microfilariae of *O. ochengi*, Ivermectin (LC_{50} = 0.13 mg/mL) was shown as the most potent, followed by hydro-ethanolic extracts of the roots (LC_{50} = 0.24 mg/mL), leaves (LC_{50} = 0.27 mg/mL) and barks (LC_{50} = 0.31 mg/mL) of *C. procera*.

Among the aqueous extracts of *C. procera*, the males of *O. ochengi* were more sensitive to the bark aqueous extract (LC_{50} = 0.23 mg/mL) compared to the aqueous extracts of the leaves (LC_{50} = 0.32 mg/mL) and roots (LC_{50} = 0.43 mg/mL) of the plant as well as Ivermectin (LC_{50} = 0.40 mg/mL). On the females of *O. ochengi*, the root aqueous extract (LC_{50} = 0.26 mg/mL) also appeared as the most toxic compared to those of the leaves (LC_{50} = 0.32 mg/mL) and of the barks (LC_{50} = 0.45 mg/mL) of the plant as well as Ivermectin (LC_{50} = 0.55 mg/mL). On the microfilariae of *O. ochengi*, Ivermectin (LC_{50} = 0.13 mg/mL) was most effective followed by the root aqueous extract (LC_{50} = 0.24 mg/mL), leaf extract (LC_{50} = 0.27 mg/mL) and bark extract (LC_{50} = 0.31 mg/mL) of *C. procera*.

3.4.3. *Effect of Faidherbia albida extracts against wild and resistant strains of Caenorhabditis elegans*

Fig. 3 presents the nematocidal activity of the hydro-ethanolic (Fig. 3A and B) and aqueous (Fig. 3C and D) extracts of the leaves, barks, and roots of *F. albida* on the wild (Fig. 3A and C) and resistant (Fig. 3B and D) strains of *C. elegans*. Generally, these extracts and the reference drug induced a significant mortality of the *C. elegans* strains and this activity increased significantly with the gradual increase of the concentrations of the plant extracts and the drug tested.

Considering the LC_{50} values obtained (Table 6), the wild strain of *C. elegans* was more sensitive to Ivermectin (LC_{50} = 0.12 mg/mL) and the bark hydro-ethanolic extract (LC_{50} = 0.19 mg/mL) of *F. albida* compared to the roots (LC_{50} = 0.29 mg/mL) and leaves (LC_{50} = 0.31 mg/mL) of the plant. However, the leaf hydro-éthanolic extract (LC_{50} = 0.13 mg/mL) of the plant were most effective against *C. elegans* resistant strain, compared to the hydro-ethanolic extract of the barks (LC_{50} = 0.28 mg/mL) and roots (LC_{50} = 0.25 mg/mL) of the plant as well as Ivermectin (LC_{50} = 0.31 mg/mL). *C. elegans* wild strain was also more sensitive to Ivermectin (LC_{50} = 0.12 mg/mL) compared to the aqueous extracts of the barks (LC_{50} = 0.42 mg/mL), leaves (LC_{50} = 0.45 mg/mL) and roots (LC_{50} = 0.48 mg/mL) of *F. albida*. Similarly, *C. elegans* resistant strain was sensitive to Ivermectin (LC_{50} = 0.31 mg/mL) compared to the aqueous extracts of the parts of *F. albida*.

3.4.4. *Effect of Calotropis procera extracts against wild and resistant strains of Caenorhabditis elegans*

Fig. 4 presents the nematocidal activity of the hydro-ethanolic (Fig. 4A and B) and aqueous (Fig. 4C and D) extracts of the leaves, barks, and roots of *C. procera* on the wild (Fig. 4A and C) and resistant (Fig. 4B and D) strains of *C. elegans*. In general, the extracts of

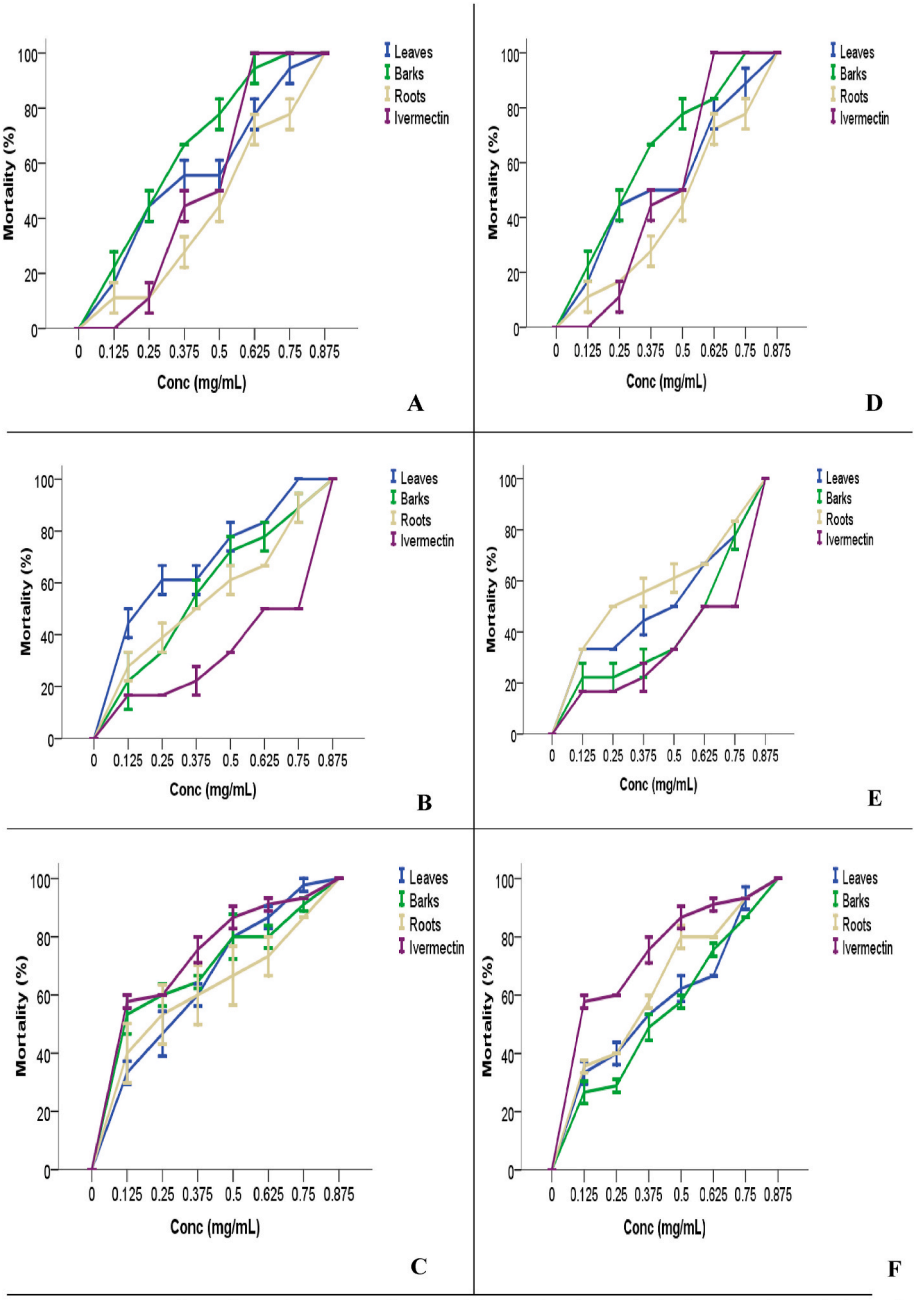

Fig. 2. Mortality of *Onchocerca ochengi* adults males (A and D), females (B and E), and microfilariae (C and F) treated with hydro-ethanolic extracts (A, B, and C) and aqueous extracts (D, E, and F) of the leaves, barks, and roots of *Calotropis procera.* In each graph, concentration 0 mg/mL represents the negative control in which no plant extract is added.

C. procera and the reference drug of induced, concentration-dependent significant mortality of the two strains of *C. elegans.*

LC_{50} values (Table 7) showed that the wild strain of *C. elegans* (LC_{50} = 0.12 mg/mL) was more sensitive to Ivermectin compared to the hydro-ethanolic extracts of the barks (LC_{50} = 0.36 mg/mL), the roots (LC_{50} = 0.36 mg/mL) and the leaves (LC_{50} = 0.42 mg/mL) of *C. procera*. However, *C. elegans* resistant strain was slightly more sensitive to ivermectin (LC_{50} = 0.31 mg/mL) compared to the hydro-ethanolic extracts of the parts of *C. procera.*

Concerning the aqueous extracts of *C. procera* (Table 7), the wild strain of *C. elegans* was more sensitive to Ivermectin (LC_{50} = 0.12 mg/mL) compared to the root aqueous extract (LC_{50} = 0.32 mg/mL), aqueous bark extract (LC_{50} = 0.38 mg/mL) and aqueous leaf extract (LC_{50} = 0.47 mg/mL) of the plant. In the same way, Ivermectin (LC_{50} = 0.31 mg/mL) was more toxic against *C. elegans* resistant strain compared to the aqueous extracts of the roots (LC_{50} = 0.44 mg/mL), the barks (LC_{50} = 0.54 mg/mL) and of the leaves (LC_{50} =

Table 5
LC_{50} and LC_{95} (mg/mL) of hydro-ethanolic and aqueous extracts of the leaves, barks, and roots of *C. procera* against males, females, and microfilariae of *O. ochengi*.

Extracts	Parasite Stages	Plant parts	Slope ± SE	R^2	LC_{50} (CI 95%)	LC_{95} (CI 95%)	χ^2
Hydro-ethanolic	Males	Leaves	2.95 ± 0.12	0.90	0.30 (0.25–0.35)	1.10 (0.85–1.64)	160.97***
		Barks	3.50 ± 0.13	0.88	0.24 (0.21–0.28)	0.73 (0.61–0.94)	127.16***
		Roots	3.58 ± 0.14	0.91	0.44 (0.36–1.53)	1.29 (0.95–2.31)	327.00***
	Females	Leaves	2.23 ± 0.11	0.87	0.18 (0.12 ± 0.23)	1.00 (0.73–1.68)	147.22***
		Barks	2.87 ± 012	0.88	0.29 (0.24–0.34)	1.10 (0.85–1.68)	172.87***
		Roots	2.35 ± 0.11	0.92	0.30 (0.23–0.35)	1.49 (1.06–2.68)	156.93***
	Microfilariae	Leaves	2.77 ± 0.12	0.88	0.23 (0.18–0.27)	0.90 (0.71–1.29)	139.73***
		Barks	1.71 ± 0.11	0.83	0.15 (0.08–0.20)	1.35 (0.91–2.86)	122.49***
		Roots	1.87 ± 0.11	0.71	0.21 (0.13–0.28)	1.63 (1.01–4.76)	213.31***
Aqueous	Males	Leaves	2.79 ± 0.12	0.91	0.32 (0.26–0.37)	1.25 (0.90–1.92)	151.60***
		Barks	3.20 ± .013	0.90	0.25 (0.21–0.28)	0.83 (0.69–1.05)	98.39***
		Roots	3.42 ± 0.13	0.92	0.43 (0.36–0.51)	1.32 (0.99–2.24)	260.89***
	Females	Leaves	1.99 ± 0.11	0.90	0.32 (0.24–0.40)	2.18 (1.36–5.51)	173.28***
		Barks	2.37 ± 0.11	0.82	0.45 (0.35–0.60)	2.25 (1.32–7.76)	320.65***
		Roots	1.93 ± 0.11	0.91	0.26 (0.19–0.31)	1.84 (1.25–3.62)	115.75***
	Microfilariae	Leaves	2.24 ± 0.11	0.93	0.27 (0.21–0.33)	1.48 (1.04–2.81)	163.33***
		Barks	2.58 ± 0.12	0.96	0.31 (0.26–0.37)	1.37 (1.03–2.18)	139.35***
		Roots	2.51 ± 0.12	0.94	0.24 (0.19–0.28)	1.08 (0.85–1.54)	101.85***
Ivermectin	Males	–	7.14 ± 0.28	0.90	0.40 (0.36–0.44)	0.68 (0.61–0.82)	182.28***
	Females	–	2.36 ± 0.12	0.79	0.55 (0.44–0.74)	2.74 (1.59–9.50)	267.09***
	Microfilariae	–	1.97 ± 0.12	0.89	0.13 (0.09–0.16)	0.90 (0.71–1.32)	71.73***

SE= Standard Error; R^2 = Coefficient of determination; LC = Lethal concentration; CI= Confident interval; χ^2 = Chi-square; ***$P < 0.001$.

0.56 mg/mL) of the plant.

4. Discussion

Plant based-medicines in decoction, infusion, maceration, or trituration forms were used and are still in use for the treatment of helminth infections. According to Ataba et al. [48], medicinal plants for anthelminthic purposes seemed to be less or no toxic for the patients, present less or no adverse effects, and are biodegradable, and environmentally safe after their use. These virtues justify their studies to discover alternate drugs resulting from natural plant products for onchocerciasis treatment since traditional medicine uses them already for longtime ago [17].

Subcutaneous onchocerciasis is a parasitic disease caused by *O. volvulus*. The cattle parasite *O. ochengi* is the species known as being closest to *O. volvulus* and shares also the same vector, the black fly *Simulium damnosum*. The system of *O. ochengi* fits the critical niche between laboratory studies using an animal model and field evaluation to control onchocerciasis in human populations. The bovine model of *O. ochengi* approaches much more human onchocerciasis with the nodules resembling narrowly those generated by *O. volvulus* [49].

In this present work, the aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of *Faidherbia albida* and *Calotropis procera* caused each, significant concentration-dependent mortality of males, females, and microfilariae of *O. ochengi*. Indeed, the studied plants were the subject of several previous studies and appeared effective against the parasites. Thus, the bark aqueous extract of *F. albida* was reported to be very effective against *Trypanosoma brucei* [50]. In Nigeria, studies undertaken by Shobowale et al. [51] showed that the aqueous and ethanolic extracts of the leaves of *C. procera* inhibit significantly some pathogenic bacteria and fungi. Furthermore, the nematocidal activity of *C. procera* would be due to the presence of active compounds such as calactin, mudarin, and calotropain with high bactericidal activity as reported by Kareem et al. [52]. In addition, several previous studies reported *anti*--Onchocerca and anthelminthic activities of the extracts of *Craterispermum laurinum* and *Morinda lucida* [53]; *Piliostigma thonningii, Ocimum gratissimum, Nauclea latifolia* and *Alstonia boonei* [54]; *Homalium Africanum* [12]; *Annona senegalensis, Anogeissus leiocarpus, Euphorbia hirta, Parquetina nigrescens* and *Khaya senegalensis* [43]. Nyasse et al. [55] reported that polycarpol isolated from *Polyalthia suaveolens* (Annonaceae) and acid 3-*O*-acetyl aleuritolic from *Discoglypremna caloneura* (Euphorbiaceae) showed significant inhibiting activity on the viability of the adult male worms of *Onchocerca gutturosa*. Phytochemical constituents such as polyphenols, tannins and compounds like voacamine, polycarpol, voacangine, ellagic acid, gentisic acid, gallic acid, (−)-epigallocatechin 3-*O*-gallate, 3-*O*-acetyl aleuritolic acid were reported to possess strong activity against onchocerca [17]. Dikti et al. [14] also reported high anti-Onchocerca and anti-Caenorhabditis activity of some and Some Proanthocyanidin Derivatives (+)-catechin-3-*O*-gallate, (−)-epi-catechin-3-*O*-gallate, (+)-gallocatechin, (−)-epigallocatechin, and (−)-epigallocatechin-3-*O*-gallate from the Fruits of *Acacia nilotica*. Studies conducted by Cho-Ngwa et al. [12] on *O. ochengi* showed a significant microfilaricidal activity of the leaf hexane extract of *Homalium africanum* (Salicaceae), leaf and root hexane extracts of *Margaritaria discoidea* (Euphorbiaciaea), leaf methylene chloride extract of *H. africanum* and *M. discoidea*. Using the cattle parasite *O. ochengi*, Ndjonka et al. [43] reported a significant microfilaricidal activity of the bark ethanolic of *Anogeissus leiocarpus* (Combretaceae) and *Khaya senegalensis* (Meliaceae) and also the leaf ethanolic extracts of *K. senegalensis* and *Euphorbia hirta* (Euphorbiaceae), while leaf aqueous extracts of *Parquetina nigrescens* (Asclepiadaceae) and *Annona senegalensis* (Annonaceae) showed a moderate effect on the worms' viability. The same tendencies were also recorded

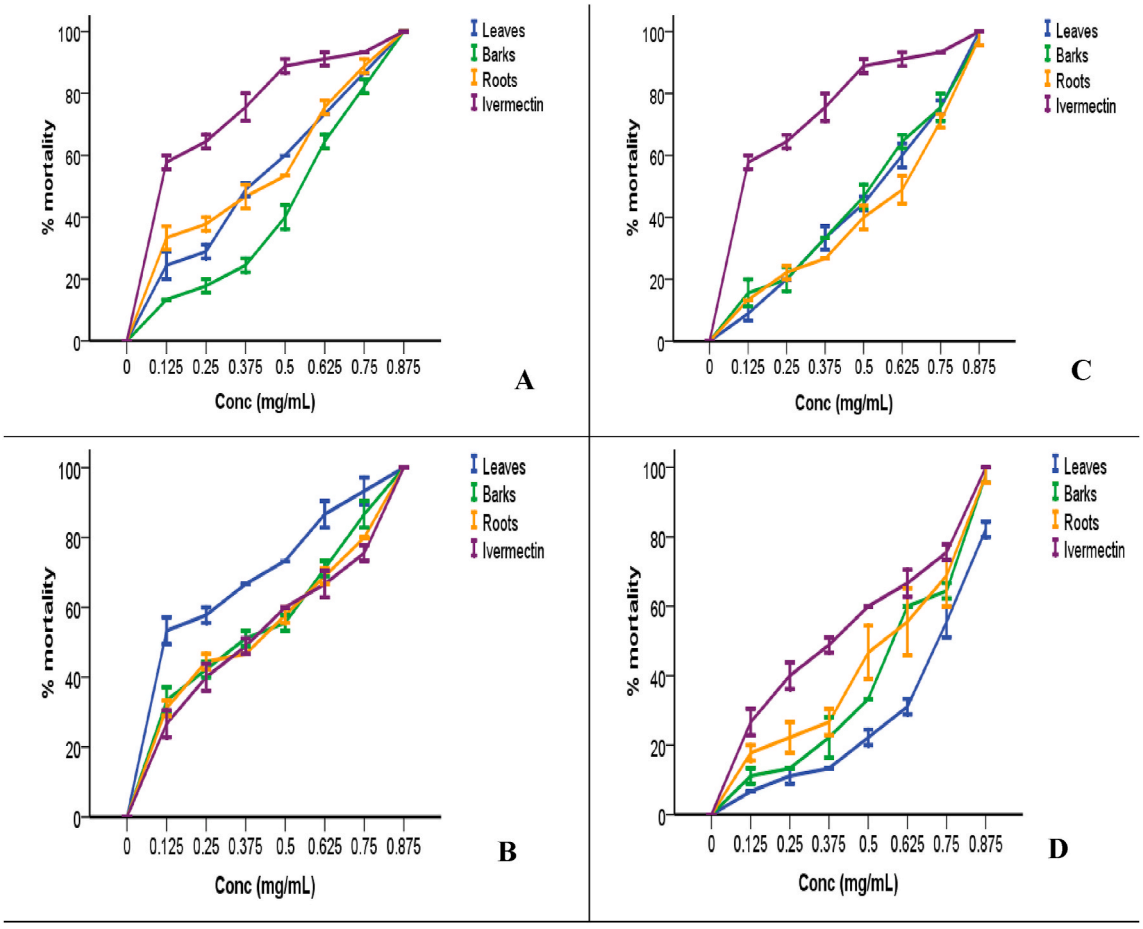

Fig. 3. Mortality of *Caenorhabditis elegans* wild strain (A and C) and resistant strain (B and D) treated with hydro-ethanolic extracts (A and B) and aqueous extracts (C and D) of the leaves, barks, and roots of *Faidherbia albida.* In each graph, concentration 0 mg/mL represents the negative control in which no plant extract is added.

Table 6
LC_{50} and LC_{95} (mg/mL) of hydro-ethanolic and aqueous extracts of the leaves, barks, and roots of *F. albida* against wild and resistant strains of *C. elegans*.

Extracts	Strains	Plant parts	Slope±SE	R^2	LC_{50} (CI 95%)	LC_{95} (CI 95%)	χ^2
Hydro-ethanolic	Wild	Leaves	2.26 ± 0.12	0.98	0.32 (0.25–0.37)	1.34 (1.03–2.01)	121.75***
		Barks	3.24 ± 0.14	0.94	0.19 (0.37–0.52)	1.43 (1.06–2.42)	224.19***
		Roots	2.25 ± 0.11	0.95	0.29 (0.22–0.35)	1.57 (1.08–3.06)	170.23***
	Resistant to Ivermectin	Leaves	1.85 ± 0.11	0.94	0.13 (0.8–0.18)	1.08 (0.79–1.85)	104.20***
		Barks	2.11 ± 0.11	0.94	0.28 (0.21–0.34)	1.69 (1.16–3.28)	140.85***
		Roots	2.03 ± 0.11	0.95	0.25 (0.19 (0.30)	1.80 (1.30–1.20)	116.25***
Aqueous	Wild	Leaves	3.25 ± 0.13	0.97	0.45 (0.39–0.51)	1.44 (1.12–2.11)	138.21***
		Barks	2.83 ± 0.12	0.96	0.42 (0.36–0.50)	1.62 (1.19–2.73)	166.56***
		Roots	2.69 ± 0.12	0.92	0.48 (0.40–0.58)	1.98 (1.37–3.84)	180.65***
	Resistant to Ivermectin	Leaves	2.89 ± 0.15	0.84	0.70 (0.59–0.88)	2.60 (1.71–5.82)	173.75***
		Barks	3.08 ± 0.13	0.91	0.47 (0.39–0.55)	1.76 (1.24–3.32)	222.97***
		Roots	2.50 ± 0.12	0.84	0.45 (0.36–0.56)	2.06 (1.33–4.98)	238.35**
Ivermectin	Resistant to Ivermectin		2.13 ± 0.11	0.94	0.31 (0.25–0.37)	1.85 (1.30–3.30)	111.63***
	Wild		1.96 ± 0.12	0.89	0.12 (0.09–0.15)	0.87 (0.69–1.20)	58.97***

SE= Standard Error; R^2 = Coefficient of determination; LC = Lethal concentration; CI= Confident interval; χ^2 = Chi-square; ***P < 0.001.

when *C. elegans*, an alive-free model and more adapted for research on parasites of the nematode was used. Moreover, certain plant extracts assessed in this work showed high toxicity not only against the wild strain of *C. elegans* but also against the resistant strain of the parasite. Ndjonka et al. [56] reported also high toxicity of *A. leiocarpus* extract (Combretaceae) against *O. ochengi* and both wild and resistant strains of *C. elegans*.

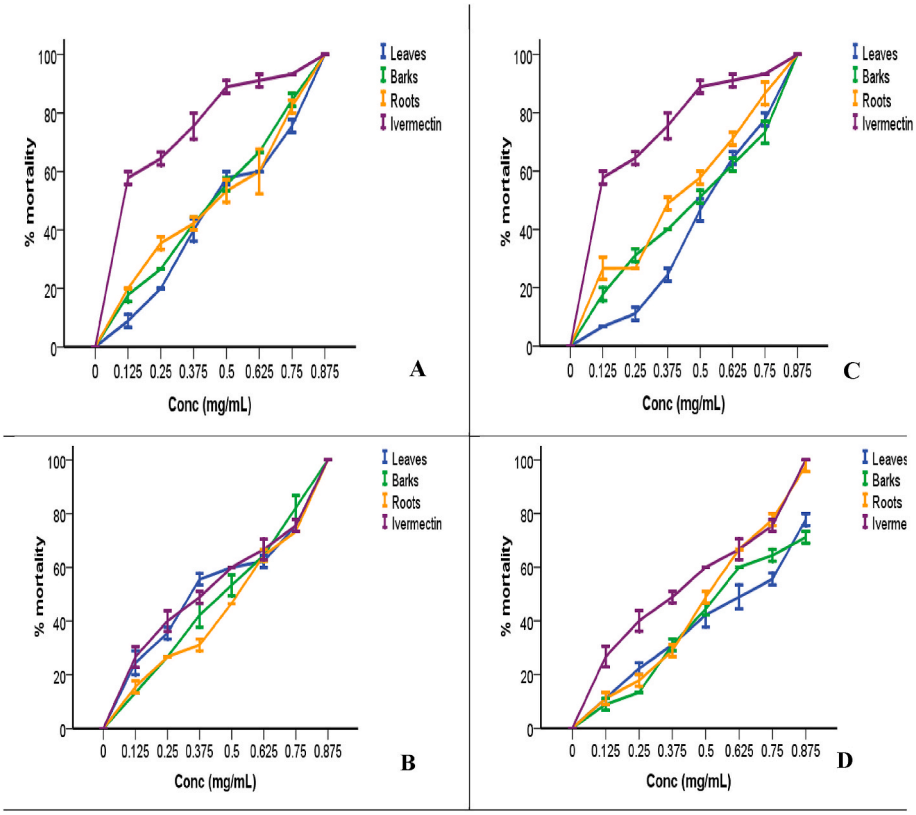

Fig. 4. Mortality of *Caenorhabditis elegans* wild strain (A and C) and resistant strain (B and D) treated with hydro-ethanolic extracts (A and B) and aqueous extracts (C and D) of the leaves, barks, and roots of *Calotropis procera.* In each graph, concentration 0 mg/mL represents the negative control in which no plant extract is added.

Table 7
LC_{50} and LC_{95} (mg/mL) of hydro-ethanolic and aqueous extracts of the leaves, barks, and roots of *C. procera* against wild and resistant strains of *C. elegans*.

Extracts	Strains	Plant parts	Slope±SE	R^2	LC_{50} (CI 95%)	LC_{95} (CI 95%)	χ^2
Hydro-ethanolic	Wild	Leaves	3.24 ± 0.13	0.97	0.42 (0.37–0.47)	1.35 (1.09–1.84)	106.07***
		Barks	2.84 ± 0.12	0.99	0.36 (0.31–0.42)	1.39 (1.08–2.04)	120.13***
		Roots	2.46 ± 0.11	0.95	0.36 (0.29–0.42)	1.67 (1.19–2.66)	151.23***
	Resistant to Ivermectin	Leaves	2.20 ± 0.11	0.92	0.32 (0.26–0.38)	1.80 (1.29–3.14)	113.01***
		Barks	2.98 ± 0.12	0.97	0.38 (0.33–0.43)	1.37 (1.08–1.96)	115.45***
		Roots	2.73 ± 0.12	0.96	0.37 (0.31–0.43)	1.68 (1.23–2.79)	147.30***
Aqueous	Wild	Leaves	3.97 ± 0.15	0.95	0.47 (0.41–0.53)	1.22 (0.99–1.71)	171.11***
		Barks	2.49 ± 0.11	0.97	0.38 (0.33–0.45)	1.77 (1.29–2.96)	126.07***
		Roots	2.55 ± 0.11	0.97	0.32 (0.26–0.38)	1.43 (1.06–2.36)	149.94***
	Resistant to Ivermectin	Leaves	2.13 ± 0.12	0.93	0.56 (0.50–0.64)	3.36 (2.40–5.49)	51.18***
		Barks	2.55 ± 0.12	0.95	0.54 (0.50–0.58)	2.40 (1.96–3.11)	30.75***
		Roots	3.26 ± 0.13	0.97	0.44 (0.38–0.49)	1.40 (1.10–2.02)	136.20***
Ivermectin	Resistant to Ivermectin			0.94	0.31 (0.25–0.37)	1.85 (1.30–3.30)	111.63***
	Wild			0.89	0.12 (0.09–0.15)	0.87 (0.69–1.20)	58.97***

SE= Standard Error; R^2 = Coefficient of determination; LC = Lethal concentration; CI= Confident interval; χ^2 = Chi-square; ***P < 0.001.

In this present investigation, the nematocidal effectiveness of the plant extracts varied significantly with not only the solvents used for extraction, but also with the various parts of each plant. Similar tendencies were also observed since tested at 20 μg/mL, 100% mortality of *O. ochengi* was recorded with the methanolic and methanol-methylene chloride extracts of the leaves, barks, and roots of *Lophira lanceolate* after 72 h; with their respective LC_{50} values of 9.76 μg/mL, 8.05 μg/mL and 6.39 μg/mL for with methanol extract and of 9.45 μg/mL, 7.95 μg/mL and 6.39 μg/mL for methanol-methylene chloride extract [15].

In this present investigation, the aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of *F. albida* and *C. procera* also caused each, significant concentration-dependent mortality of both wild and resistant strains of *C. elegans*. Similarly, on the wild strain of *C. elegans*, ethanolic and methanol-methylene chloride extracts of *Lophira lanceolata* exhibited moderate mortality with respective

LC_{50} values after 72 h of 1890 μg/mL and 1200 μg/mL for the root barks and 1000 μg/mL and 2030 μg/mL for the trunk barks [15]. Indeed, the anthelminthic resistance of the pathogenic helminths in the human being and animals was spread in prevalence in severity at a point where resistance to the multiple drugs against the three great anthelminthic classes (benzimidazoles, imidazothiazoles, and lactones macrocyclic) became a complete phenomenon of the parasitic nematodes at the human and in animals. Like Ivermectin, the phytochemical compounds resulting from hydro-ethanolic and aqueous extracts of the leaves, barks, and roots of the studied plants would have a strong affinity for the channels chlorides glutamate-dependent on the nervous and muscular cells of the microfilariae and the adults of *O. ochengi*. Indeed, the binding of these phytochemicals might increase the cell membrane permeability to chloride ions, resulting in the hyperpolarization of the parasite cells, conducting to the paralysis and death of the microfilariae and adults of *O. ochengi* [57].

5. Conclusion

The present study revealed the toxic effects of concentration-dependent *in vitro* of aqueous and hydro-ethanolic extracts of the leaves, barks, and roots of *F. albida* and *C. procera.* These extracts may possess at the same time significant macro/microfilaricidal activities. Among the plant extracts of the 2 plants tested, the bark hydro-ethanolic extract of *F. albida* was the most effective against the microfilariae, males and females of *O. ochengi* as well as the strains of *C. elegans.* This study validates the use of these plants by traditional health practitioners in the management of the disease and can constitute a new source for the development of an effective drug to control both sensitive and resistant strains of nematodes. Furthermore, the bark hydro-ethanolic extract of *F. albida* should be submitted to bio-guided fractionation and isolation of the active compounds responsible of the anthelminthic activity.

Author contribution statement

Amina Mamat: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Younoussa Lame: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fanta Adeline; Nguezeye Yvette: Performed the experiments.

Okah-Nnane Herman: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Bitja-Nyom Roger: Conceived and designed the experiments; Analyzed and interpreted the data.

Ndjonka Dieudonné: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

The authors are grateful to the Veterinary Research Laboratory of the Institute of Agricultural Research for Development (IRAD), Wakwa, Ngaoundere, Cameroon where the anti-helminthic assays were conducted. The authors are also thankful to the laboratory of Phytochemistry of the Institute of Medical Research and Medicinal Plants Studies (IMPM) where plants were extracted.

References

[1] S.L. James, D. Abate, K.H. Abate, S.M. Abay, C. Abbafati, N. Abbasi, H. Abbastabar, F. Abd-Allah, J. Abdela, A. Abdelalim, Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017, Lancet 392 (2018) 1789–1858, https://doi.org/10.1016/S0140-6736(18)32279-7.

[2] C.D. Enk, Onchocerciasis - river blindness, Clin. Dermatol. 24 (2006) 176–180, https://doi.org/10.1016/j.clindermatol.2005.11.008.

[3] WHO (World Health Organization), Onchocerciasis fact sheet. https://www.who.int/en/news-room/fact-sheets/detail/onchocerciasis, 2022.

[4] WHO (World Health Organization), Elimination of Human Onchocerciasis: Progress Report, 2019-2020, WHO, Geneva, 2020. https://www.who.int/publications/i/item/who-wer9545-545-554.

[5] H.G. Zoure, M. Noma, A.H. Tekle, U.V. Amazigo, P.J. Diggle, E. Giorgi, J.H. Remme, The geographic distribution of onchocerciasis in the 20 participating countries of the African Programme for Onchocerciasis Control: (2) pre-control endemicity levels and estimated number of infected, New. Pub. Med. 7 (2014) 326–331, https://doi.org/10.1186/1756-3305-7-326.

[6] I. Ejiakor, E. Achigbu, O. Onyia, O. Edema, U.N. Florence, Impact of visual impairment and blindness on quality of life of patients in Owerri, Imo State, Nigeria. Middle East Afr, J. Ophthalmol. 30 (2019) 127–132, https://doi.org/10.4103/meajo.MEAJO_256_18.

[7] D. Oguttu, E. Byamukama, C.R. Katholi, P. Habomugisha, C. Nahabwe, M. Ngabirano, H.K. Hassan, T. Lakwo, M. Katabarwa, F.O. Richards, T.R. Unnasch, Serosurveillence to monitor onchocerciasis elimination: the Uganda experience, Am. J. Trop. Med. Hyg. 90 (2014) 339–345, https://doi.org/10.4269/ajtmh.13-05 4 6.

[8] WHO (World Health Organization), Progress towards eliminating onchocerciasis in the WHO Region of the Americas: verification of elimination of transmission in Mexico, (43) 90 (2015) 577–581. WHO, https://apps.who.int/iris/handle/10665/254517.

[9] T.E. Makou, T.F. Guemgne, G.A. Gamago, P.J. Wabo, T.F. F Djuikwo, Prevalence of onchocerciasis after seven years of continuous community-directed treatment with ivermectin in the Ntui health district, Centre region, Cameroon, Pan Afr. Med. J. 36 (2020) 1–8, https://doi.org/10.11604/pamj.2020.36.180.20765.

[10] WHO (World Health Organization), Guidelines for stopping mass drug administration and verifying elimination of human onchocerciasis, in: Criteria and Procedures, Geneve, 2017. License/CCBY-NC-SA3.0IGO.ISBN 978-92-4-251001-0.

[11] A. Sofowora, E. Ogunbodede, A. Onayade, The role and place of medicinal plants in the strategies for disease prevention, Afr. J. Tradit., Complementary Altern. Med. 12 (2013) 210–229, https://doi.org/10.4314/ajtcam.v10i5.2.

[12] F. Cho-Ngwa, M. Abongwa, N.M. Ngemenya, K.D. Nyongbela, Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca ochengi*, BMC Compl. Alternative Med. 10 (2010) 62–65, https://doi.org/10.1186/1472-6882-10-62.

[13] F. Cho-Ngwa, E. Monya, B.K. Azantsa, P.T.F. Manfo, S.B. Babiaka, J.A. Mbah, M. Samje, Filaricidal activities on *Onchocerca ochengi* and *Loa loa*, toxicity and phytochemical screening of extracts of *Tragia benthami* and *Piper umbellatum*, BMC Compl. Alternative Med. 16 (2016) 326, https://doi.org/10.1186/s12906-016-1319-2.

[14] J. Dikti, J. Kalmobe, D. Boursou, T. J Schmidt, E. Liebau, D. Ndjonka, Anti-onchocerca and Anti-Caenorhabditis activity of a hydro-alcoholic extract from the fruits of *Acacia nilotica* and some Proanthocyanidin derivatives, Molecules 22 (2017) 748–750, https://doi.org/10.3390/molecules22050748.

[15] J. Kalmobé, D. Ndjonka, D. Boursou, V.J. Dikti, E. Liebau, 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 Compl. Alternative Med. 17 (2017) 404–410, https://doi.org/10.1186/s12906-017-1904-z.

[16] T.M.H. Ndouwe, D. Ndjonka, R. Mimpfoundi, Anthelmintic activity, acute toxicity of *Anacardium occidentale* L. (Anacardiaceae) on *Onchocerca ochengi* and *Caenorhabditis elegans*, Asian J. Med. Health 5 (2017) 1–12, https://doi.org/10.9734/AJMAH/2017/34454.

[17] D. Ndjonka, B. Djafsia, L. Eva, Review on medicinal plants and natural compounds as anti-Onchocerca agents, Parasitol. Res. 117 (2018) 1–17, https://doi.org/10.1007/s0 0436-018-6003-7.

[18] G.S. Cavalcante, S.M. Morais, W.P. Andre, W.L. Ribeiro, A.L. Rodrigues, F.C. De Lira, Chemical composition and in vitro activity of *Calotropis procera* (Ait.) latex on *Haemonchus contortus*, Vet. Parasitol. 5 (2016) 226–229, https://doi.org/10.1016/j.vetpar. 2016.06.012.

[19] B.D. Wadhwani, D. Mali, P. Vyas, R. Nair, P. Khandelwal, A review on phytochemical constituents and pharmacological potential of *Calotropis procera*, RSC Adv. 11 (2012) 35854–35878, https://doi.org/10.1039/d1ra06703f.

[20] D.E. Tsala, N. Nga, B.N. Thiery, M.T. Bienvenue, D. Theophile, Evaluation of the antioxidant activity and the healing action of the ethanol extract of *Calotropis procera* bark against surgical wounds, J. Intercult. Ethnopharm. 42 (2015) 64–69, https://doi.org/10.5455/jice.20141211071136.

[21] A.D. Sayed, N.H. Mohamed, M.A. Ismail, W.M. Abdel-Mageed, A. Shoreit, Antioxidant and antiapoptotic activities of *Calotropis procera* latex on Catfish (*Clarias gariepinus*) exposed to toxic 4-nonylphenol, Ecotoxicol. Environ. Saf. 128 (2016) 189–194, https://doi.org/10.1016/j.ecoenv.2016.02.023.

[22] A.K. Ashishkumar, P.A. Meghsham, V.N. Ashwin, V.P. Uday, In vivo study of pharmacological activities of *Calotropis procera*: an overview, Int. J. Res. Ayurveda Pharm. 13 (2022) 44–49, https://doi.org/10.7897/2277-4343.130356.

[23] A.B. Gurung, M.A. Ali, A. Bhattacharjee, M. AbulFarah, F. Al-Hemaid, F.M. Abou-Tarboush, Molecular docking of the anticancer bioactive compound proceraside with macromolecules involved in the cell cycle and DNA replication, Genet. Mol. Res. 15 (2016) 1–7, https://doi.org/10.4238/gmr.15027829.

[24] S. Quazi, K. Mathur, S. Arora, *Calotropis procera*: an overview of its phytochemistry and pharmacology, Indian J. Drugs 1 (2013) 63–69. https://drugresearch.in/pdf/IJOD-7-kumkum.pdf.

[25] P. Chaudhary, M.V. Ramos, S. Vasconcelos-Mda, V.L. Kumar, Protective effect of high molecular weight protein sub-fraction of *Calotropis procera* latex in monoarthritic rats, Phcog. Mag. 12 (2016) 147–151, https://doi.org/10.4103/0973-1296.182151.

[26] S. Gupta, B. Gupta, K. Kapoor, P. Sharma, The ethnopharmacological potential of *Calotropis procera*: an overview, Int. Res. J. Pharm. 3 (2012) 19–22. https://www.irjponline.com/admin/php/uploads/1521_pdf.pdf.

[27] R.M. Aliyu, M.B. Abubakar, A.B. Kasarawa, Y.U. Dabai, N. Lawal, M.B. Bello, A.Y. Fardami, Efficacy and phytochemical analysis of latex of *Calotropis procera* against selected dermatophytes, J. Intercult. Ethnopharmacol. 4 (2015) 314–317, https://doi.org/10.5455/jice.20151012012909.

[28] G. Parihar, N. Balekar, Immunomodulating potential of *Calotropis procera* (Ait.) root bark ethanolic extract on experimental animal, J. Adv. Pharm. Educ. Res. 4 (2014) 289–297. https://japer.in/storage/models/article/QNA3XtkKhcZs4igJ44Yl13RCuOOEcjLUfkInRAalxCZaQ09JPWiDGxe6ATRe.

[29] G. Parihar, N. Balekar, *Calotropis procera*: a phytochemical and pharmacological review, TJPS 40 (2016) 115–131. https://www.thaiscience.info/journals/Article/TJPS/10984244.

[30] D.C. Nascimento, M.T. Ralph, J.E. Batista, D.M. Silva, M.A. Gomes-Filho, N.M. Alencar, Latex protein extracts from *Calotropis procera* with immunomodulatory properties protect against experimental infections with Listeria monocytogenes, Phytomedicine 23 (2016) 745–753, https://doi.org/10.1016/j.phymed.2016.03.012.

[31] B.B. Tchebemou, D. Soh, T.Z.E. Nkewngoua, D.Y.O. Nganso, M.T. Emmanuel, A.M. Kerstin, B. Nyasse, Phytochemical activity of *Faidherbia albida* (Mimosaceae) roots and leaves, Int. J. Pharmaceut. Sci. Rev. Res. 65 (2020) 124–130, https://doi.org/10.47583/ijpsrr.2020.v65i01.018.

[32] W. Usman, M. Jada, Z. Ahmed, Antimicrobial activity of stem bark of *Faidherbia albida*, J. Pharm. Res. Int. 3 (2013) 786–794, https://doi.org/10.9734/BJPR/2013/3209.

[33] O.A. Salawu, A.Y. Tijani, H. Babayi, A.C. Nwaeze, R.A. Anagbogu, V.A. Agbakwuru, Antimalarial activity of ethanolic stem bark extract of *Faidherbia albida* (Del) A. Chev (Mimosoidae) in mice, Arch. Appl. Sci. Res. 2 (2010) 261–268. https://www.scholarsresearchlibrary.com/archive.html.

[34] H.G. Ahmed, A.E. Sayed, H.A. Abdel-razik, M.A. Nadia, Antidiabetic, antihyperlipidemic, and antioxidant activities of *Acacia albida* in streptozotocin-induced diabetes in rats and its metabolites, E. J. Chem. 15 (2020) 337–348, https://doi.org/10.21608/EJCHEM.2019.18863.2160.

[35] J. P Mccall, H. Townson, A.J. Trees, Morphometric differentiation of the *Onchocerca volvulus* and *O. ochengi* infective larvae, Trans Roy Soc Med Hyg 86 (1992) 63–65, https://doi.org/10.1016/0035-9203(92)90443-G.

[36] A.J. Trees, *Onchocerca ochengi*: mimic, model, or modulator of *O. volvulus*? Parasitol. Today 8 (1992) 337–339.

[37] V.M. Nigon, M.A. Félix, History of Research onC. Elegans and Other Free-Living Nematodes as Model Organisms, in: Worm Book, 2017, pp. 1–84, https://doi.org/10.1895/WORMBOOK.1.181.1.

[38] J.A. Dent, M.M. Smith, D.K. Vassilatis, L. Avery, The genetics of ivermectin resistance in *Caenorhabditis elegans*, PMID: 10716995; PMCID: PMC15988, Proc. Natl. Acad. Sci. USA 97 (2000) 2674–2679, https://doi.org/10.1073/pnas.97.6.2674.

[39] M.K. Witabouna, M. Vargas, J. Keiser, Anthelmintic activity of medicinal plants used in Côte d'Ivoire for treating parasitic diseases, Parasitol. Res. 110 (2012) 2351–2362, https://doi.org/10.1007/s00436-011-2771-z.

[40] A. Sofowora, Medicinal Plants and Traditional Medicines in Africa, Spectrum Books Ltd., Ibadan, 1993, pp. 191–289, https://doi.org/10.4236/ajmb.2021.114013, 2.

[41] J.B. Harbone, Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London, London, New York 10017, USA, 1998, p. 150. http://www.thomson.com.

[42] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. J. 64 (1999) 555–559, https://doi.org/10.1016/S0308-8146(98)00102-2.

[43] D. Ndjonka, C. Agyare, K. Lüersen, B. Djafsia, D. Achukwi, E.N. Nukenine, A. Hensel, L. Eva, *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes, J. Helminthol. 85 (2011) 304–312, https://doi.org/10.1017/S0022149X10000635.

[44] R.A. Smith, L. Pontiggia, C. Waterman, M. Lichtenwalner, J. Wasserman, Comparison of motility, recovery, and methyl-thiazolyl-tetrazolium reduction assays for use in screening plant products for anthelmintic activity, Parasitol. Res. 105 (2019) 1339–1343, https://doi.org/10.1007/s00436-009-1560-4.

[45] J.L. Chen, T.W.J. Steele, D.C. Stuckey, Metabolic reduction of resazurin; location within the cell for cytotoxicity assays, Biotech. Bioenergy. 115 (2018) 351–358, https://doi.org/10.1002/bit.26475.
[46] K. Han, J. Lee, G. Shanmugam, S.K. Lee, J. Jeon, Development of a redox dye-based rapid colorimetric assay for the quantitation of viability/mortality of pine wilt nematode, J. Microbiol. Biotechnol. 29 (2019) 1117–1123, https://doi.org/10.4014/jmb.1903.03005.
[47] D.J. Finney, Probit Analysis. 3. Aufl, Cambridge University Press, Cambridge 1971. XV, 333 S., 41 Rechenbeispiele, 20 Diagr., 8 Tab., 231 Lit., London, 1979, pp. 68–72, https://doi.org/10.1002/bimj.19720140111.
[48] E. Ataba, G. Katawa, M. Ritter, A.H. Ameyapoh, K. Anani, O.M. Amessoudji, P.E. Tchadié, T. Tchacondo, K. Batawila, Y. Ameyapoh, A. Hoerauf, L.E. Layland, S. D. Karou, The ethnobotanical survey, anthelmintic effects, and cytotoxicity of plants used for the treatment of helminthiasis in the Central and Kara regions of Togo, BMC Complement. Med. Ther. 20 (2020) 212, https://doi.org/10.1186/s12906-020-03008-0.
[49] D.M. Achukwi, W. Harnett, A. Renz, *Onchocerca ochengi* transmission dynamics and the correlation of *Onchocerca ochengi* microfilaria density in cattle with the transmission potential, Vet. Res. 31 (2000) 611–621, https://doi.org/10.1015/vetres/;2000144.hal-00902681.
[50] U.I. Alhaji, N.U. Samuel, M. Aminu, *In vitro* anti-trypanosomal activity, antioxidant property, and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants, Asian Pac. J. Trop. Dis. 4 (2014) 348–355, https://doi.org/10.1016/S2222-1808(14)60586-7.
[51] O.O. Shobowale, N.J. Ogbulie, E.E. Itoandon, M.O. Oresegun, S.O.A. Olatope, Phytochemical and antimicrobial evaluation of aqueous and organic extracts of *Calotropis procera* ait leaf and latex, Braz. J. Microbiol. 38 (2013) 369–380, https://doi.org/10.1016/S0189-7241(15)30059-X.
[52] S.O. Kareem, I. Akpan, O.P. Ojo, Antimicrobial activities of *Calotropis procera* on selected pathogenic micro-organisms, Afr. J. Biomed. Res. 11 (2008) 105–110. https://www. ajbrui.com.
[53] M. Samje, J. Metuge, J. Mbah, B. Nguesson, F. Cho-Ngwa, In vitro anti-*Onchocerca ochengi* activities of extracts and chromatographic fractions of *Craterispermum laurinum* and *Morinda lucida*, BMC Compl. Alternative Med. 14 (2014) 325–332, https://doi.org/10.1186/1472-6882-14-325.
[54] B.B. Fakae, A.M. Campbell, J. Barrett, I.M. Scott, P.H. Teesdale-Spittle, E. Liebau, P.M. Brophy, Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants, Phytother Res. 14 (2000) 630–634, https://doi.org/10.1002/1099-1573(200012)14:8<630::aid-ptr773>3.0.co;2-5.
[55] B. Nyasse, I. Ngantchou, J.J. Nono, B. Schneider, Antifilarial activity *in vitro* of polycarpol and 3-O-acetyl aleuritolic acid from Cameroonian medicinal plants against *Onchocerca gutturosa*, Nat. Prod. Res. 20 (2006) 391–397, https://doi.org/10.1080/14786410 600661377.
[56] D. Ndjonka, I. Ajonina-Ekoti, B. Djafsia, K. Lüersen, E. Abladam, L. Eva, *Anogeissus leiocarpus* extract on the parasite nematode *Onchocerca ochengi* and on drug-resistant mutant strains of the free-living nematode *Caenorhabditis elegans*, Vet. Parasitol. 190 (2012) 136–142, https://doi.org/10.1016/j.vetpar.2012.05.027.
[57] S. Sharmeen, M. Skrtic, M.A. Sukhai, R. Hurren, M. Gronda, X. Wang, S.B. Fonseca, H. Sun, T.E. Wood, R. Ward, M.D. Minden, R.A. Batey, A. Datti, J. Wrana, S. O. Kelley, A.D. Schimmer, The antiparasitic agent ivermectin induces chloride-dependent membrane hyperpolarization and cell death in leukemia cells, Epub 2010 Jul 19. PMID: 20644115, Blood 4 (2010) 3593–3603, https://doi.org/10.1182/blood-2010-01-262675.