European Journal of Medicinal Plants

19(3): 1-12, 2017; Article no.EJMP.33290
ISSN: 2231-0894, NLM ID: 101583475

In vivo Toxicity Study and Antifilarial Activity of Four Plants from Nord-Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ND, AM, AA and DB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AM, AA and NTMH managed the analyses of the study. Authors AM and AA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/33290
Editor(s):
(1) Ghalem Bachir Raho, Biology Department, Sidi Bel Abbes University, Algeria.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
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(4) Abdullah M. Tauheed, Ahmadu Bello University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/19467

Original Research Article

Received 8th April 2017
Accepted 6th June 2017
Published 10th June 2017

ABSTRACT

Aims: The objective of this work was to seek an alternative drug against onchocerciasis based on medicinal plants.
Study Design: Ethanolic extracts of stem barks, leaves and roots of *Detarium microcarpum*, *Guiera senegalensis*, *Trichilia emetica* and *Vitellaria paradoxa* were evaluated *in vitro* against the cattle filarial parasite *Onchocerca ochengi*, a model organism similar to *Onchocerca volvulus*.
Place and Duration of Study: The work took place at the Laboratory of Parasitology of the Institute of Agricultural Research for Development of Ngaoundere between October 2014 and February 2015.
Methodology: Adult worms were incubated in RPMI 1640 medium supplemented with antibiotics, and different concentrations of the extracts of the four plants. Mortality was registered after 24, 48

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and 72 h of incubation at 37ºC. Ivermectin and M9-DMSO were the positive and negative controls respectively.
Results: All parts of plants showed anthelmintic activities after 72 h of incubation. The Means of LC_{50} values were determined graphically and varied from 5 to 60 µg/mL after 72 h incubation. The most antifilarial activities were obtained from stem barks and leaves of *D. microcarpum* with LC_{50} of 5 and 7.9 µg/mL on adult worms respectively, while the least antifilarial activity was obtained from stem barks of *V. paradoxa* with LC_{50} of 60 µg/mL. These results show that at low concentrations, leaves and stem barks of *D. microcarpum* are effective in killing *O. ochengi* worms. Additionally, *in vivo* toxicity tests using mice showed that the four plants are not toxic.
Conclusion: The findings of the present study support the use of these plants against nematode infections by traditional healers and pastoralists in Cameroon and could represent an alternative anthelminthic for onchocerciasis treatment.

Keywords: Cattle; herbal medicine; river blindness; toxicity; treatment.

1. INTRODUCTION

Onchocerciasis is a disease caused by a parasitic nematode *Onchocerca volvulus.* The disease affects over 36 million people worldwide and is endemic in 20 African countries, in Yemen and parts of Latin America. Eighty-six million people at risk of being infected [1]. It is a human-pathogenic filarial parasite that is transmitted by blackflies of the genus *Simulium damnosum* causing a large spectrum of symptoms and is responsible of blindness [2]. Clinical manifestations are itching and eye lesions which latter lead to visual impairment and blindness [3]. In the human host, the worms live under the skin, where they form nodules at the adult stage [4]. The females of *O. volvulus* produce thousands of microfilariae per day that migrate subcutaneously throughout the human body [5].

The most used chemotherapeutic agent for treating onchocerciasis is ivermectin. This drug is the only existing drug approved for the treatment of onchocerciasis and has been the cornerstone of the Onchocerciasis Control Program (OCP) and later the African Program for Onchocerciasis Control (APOC) [6]. The administration of ivermectin in high doses is not safe [7]. It is microfilaricide, requires more than 15 years of treatment, and causes important side effects which could lead to death in cases of co-endemicity with loasis [7]. These facts with the established resistance developed by *O. volvulus* parasites in some communities in Ghana and in Sudan [8], limit its application [7] and emphasize the importance of searching for new and more effective pharmaceutical drugs to treat the disease.

One of the strategies for developing novel pharmaceutical drugs is to use natural sources such as plants for therapeutics. Isolation of pure compounds leads frequently to the loss of the activity. Owing to the lack of a laboratory host for the human parasite *O. volvulus*, model organisms are often needed. Accordingly, the bovine filarial parasite *Onchocerca ochengi* has been used in some promising antifilarial drug studies [9-13]. The two parasites are evolutionary closely related and are transmitted to their human and bovine host by the same blackfly vector *Simulium damnosum* [14].

Pure compounds isolated from *Cyperus articulates*, *Craterispermum laurinum*, *Morinda lucida* and *Acacia nilotica* have shown activities with LC_{50} concentrations ranking from 7.8 µg/ml to 46.8 µg/ml on *O. ochengi* [11-13,15].

Based on information of the regular use by traditional healers, herdsmen and pastoralists for the treatment of human and livestock parasites, we have recently carried out a study of the nematocidal activity of a group of plants. The results demonstrated the antifilarial activity of the crude extracts of *Anogeissus leiocarpus, Khaya senegalensis, Annona senegalensis* and *Euphorbia hirta* with the lowest LC_{50} value of 30 µg/ml on *O. ochengi* after 72 h [16,17]. Elsewhere, Samje et al. [13] reported IC_{100} value of 250 µg/ml with crude extracts of *Morinda lucida*. Up to present, no crude extract has shown antifilarial activity with LC_{50} value lower than 5 µg/ml on *O. ochengi* adult.

In the present study, we assessed the *in vitro* anthelminthic effect of four plants namely *Detarium microcarpum*, *Giuera senegalensis*, *Vitellaria paradoxa* and *Trichelia emetica*, with promising nematocidal activities.

2. MATERIALS AND METHODS

2.1 Plant Material and Chemicals

The plants were collected in savannas. *Detarium microcarpum*, *Vitellaria paradoxa* and *Trichelia emetica* leaves, stem barks and roots (Table 1) were collected in Wakwa (7º20' North, 13º30' East), Ngaoundéré the Adamaoua region in June 2016. *Guiera senegalensis* parts were collected in Kalfou (10º 16' 56'' North, 14º 55' 58'' East), Maroua the far-North region in August 2016.

They were identified by a botanist (Dr. Tchopsala) of the Department of Biological Sciences, University of Ngaoundere, Cameroon. A voucher specimen was deposited in the National Herbarium of Yaounde, Cameroon (6499/SRF/cam, 24348/SRF/cam, 20887/SRF/cam and 21818/SRF/cam for *Detarium microcarpum*, *Giuera senegalensis*, *Trichelia emetica* and *Vitellaria paradoxa*, respectively). Unless stated otherwise, all chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2 Preparation of Plant Extracts

Plant extracts were prepared as previously described [16]. Briefly, 10 g of the powdered material was extracted in 100 ml of ethanol (70% v/v) for 48 h at laboratory temperature (25-30 ℃), centrifuged (3,500× g, 10 min) and filtered over filter papers No. 413 (VWR International, Darmstadt, Germany). The clear filtrate was concentrated by a rotary evaporator at a temperature not exceeding 40℃ under reduced pressure, lyophilized, and the resulting powder was stored at 4ºC. After preparation dried extracts of each plant material were dissolved in growth medium containing salt (M9 medium) (3 g/l KH2PO4, 6 g/l Na2HPO4, 5 g/l NaCl and 0.25 g/l MgSO4·7H2O) and 1% dimethyl sulphoxyde (DMSO)

Table 1. Plants used for *in vitro* for antifilarial activity

Plants (family)	Parts (organ)	Biological activity and application	Extraction
Detarium microcarpum Guill. and Perr. (Caesalpiniaceae)	Leaves, bark, roots	Used for the treatment of constipation, dysentery, conjunctivitis, fever, itch, scabies, and wounds. Treatment of stomach aches, antiviral and antimicrobial activity, moderate antitumor efficiency against cancer cells of the breast. Treatment of anorexia, anemia and sexual erectile disfunction [18-20]	Ethanol
Guiera senegalensis J. F. Gmel (Combretaceae)	Leaves, bark, roots	Antiprotozoa, nematotoxic, efficient against *Plasmodium falciparum*, active on bacteria, viruses and *Trypanosoma brucei bucei* [21-22]	Ethanol
Trichelia emetica Vahl (Meliaceae)	Leaves, bark, roots	Is usefull as treatment against malaria, cough, gastric ulcer, asthma, cirrhosis, intestinal worms, hemorrhoids, mental illness, epilepsy typhoid fever, hypertension. Activity against poisoning, hepatitis, effect on prostate cancer cells, infections of the skin and mouth infections. Treatment of upset stomach, syphilis and bark is used as a purgative. Leaves serve for healing of wounds [23-26]	Ethanol
Vitellaria paradoxa C.F.Gaertn (Sapotaceae)	Leaves, bark, roots	Was shown effective against headache, for the treatment of sores, diarrhea, skin diseases, activity against salmonelosis, moderate antimalarial activity and antiproliferative effect on cancer cells [27-30].	Ethanol

to a final concentration of 50 mg/ml, centrifuged and aliquoted to determine their activity on *Onchocerca ochengi*.

2.3 Phytochemical Screening of Plant Extracts

Phytochemical content of the extracts with 4 derivatives was determined using the standard known methods. The tannins content was evaluated following the method described by Kumaran et al. [31]. Briefly, 200 µl of the sample were mixed with 35 % Na_2CO_3 (w/v) and 100 µl of Folin-Ciocalteu (FC) reagent was added. The obtained solution was homogenized by vortexing for a minute, incubated for five minutes and the absorbance read at 640 nm. The proportion of tannins was expressed as mg equivalent of gallic acid per gram of dry plant material (mg of GAE/g).

Phenolics content was estimated following the Folin-Ciocalteu principle described by Wolfe et al. [32]. The gallic acid amount is evaluated in a serie of dilution in an aqueous solution by photometric quantification. A titration curve of gallic acid is set during the experiment. Briefly, 50 µl of sample was mixed with 200 µl of 35 % Na_2CO_3, the solution was mixed manually for few second and 250 µl of 1/10 (v/v) FC reagent added. The mixture was homogenized and incubated for 30 minutes at 40ºC in dark and absorbance was read at 765 nm with a spectrophotometer (UV-Biowave Cambridge, England). Phenolics content was determined by calculation using a linear equation of the gallic acid titration curve. The content was expressed in mg equivalent of gallic acid per gram of dry plant material (mg of GAE/g).

Quantification of flavonoids was performed according to the method described by Wolfe et al. [32]. 0.1 g of the extract was mixed with 2 ml of an extraction solvent made of 140:50:10 methanol-distilled water-acetic acid, mixed manually and filtered using a Wattman paper. The filtrated was adjusted with a volume of the extraction solvent. 250 µl of the filtrate was taken to a 5 ml tube and top up to the final volume with distilled water: the obtained solution is the analysis solution. Rutin was the standard for titration: 1 ml of analysis solution was mixed with 200 µl of distilled water and 500 µl of aluminium chloride solution (400 mg of CH_3COONa anhydrous and 133 mg of $AlCl_3$) and the absorbance read at 430 nm. The flavonoids amount was expressed as mg of rutin per 100 grams of dry plant material.

Saponins were determinate using the methodology of Obadoni and Ochuko [33]. To 0.1 g of the extract, 1 ml of distilled water was added and vigorously shaken for 30 min. The height of moss was measured by a ruler and quantified like following: Saponin (mg) = [(0.432) (height of moss in cm after 5 to 10s) + 0.008] / (weight of sample in gram).

2.4 Sampling and *in vitro* Screening Assay of *O. ochengi* Adults

Nodules removed from the umbilical skin of cattle slaughtered in the local slaughterhouse were brought to the laboratory, washed, drained and sterilized with 10 % povidone iodine (Galentic Pharma, Mumbai/India) for dissection as described by Ndjonka et al. [16]. After extirpation of nodules, *O. ochengi* were extracted, isolated and washed three times with sterile phosphate-buffered saline (PBS). The adult female worms were isolated by digestion of the nodules with collagenase at 37ºC. Worms were incubated following the protocol of Borsboom et al. [34]. Briefly, six individuals (1 per 1 ml culture medium/well) were incubated with different concentrations of the plant extracts (0–0.5 mg/ml) in RPMI 1640 supplemented with penicillin/streptomycin (100 U/100 µg/ml). Assays were incubated at 37ºC and mortality was determined after 24 h, 48 h and 72 h.

2.5 *In vitro* Assays: Mortality of Worms

Adult worms were washed twice and subsequently transferred into RPMI-1640 medium supplemented with L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin and incubated at 37ºC in 24-well plates with different concentrations of the tested plant extracts. Worm mortality was checked by observation under the microscope after 24, 48 and 72 h of incubation. After shaking, immotile and fully elongated individuals were considered being dead. All tests were done in three independent duplicate determinations.

The mortality rate was expressed as the percentage of number of dead divided by the number of living swimming worms. LC_{50} values were determinate (lethal concentration of the extract required to kill 50% of the tested subjects). Results are presented as mean values

± standard error of the mean (SEM). Error bars in bar graphs are SEM.

100 mg of ivermectin (Merck sharp & Co, Rahway/USA) was dissolved in 10% DMSO in distilled water. The drug was diluted with M9 to a final concentration of 2.2 mg/ml (2.5 mM) used for the preparation of the positive control groups and M9-DMSO as negative control. The maximal final concentration of DMSO in the test is 1%.

2.6 Experimentation with Mice

Eight to twelve-week-old BALB/c mice, with an average weight between 20 to 25 g served for the experiments were purchased at LANAVET (Laboratoire National Vétérinaire, Garoua/Cameroon). Animals were housed and maintained at the Veterinary Research Laboratory of the Institute of Agricultural Research for Development, Wakwa Regional Centre, Ngaoundere, Cameroon under ambient temperatures (25ºC ± 3ºC) and 12 h light/dark cycle. The access of the animals to drinking water and rodent pellets was unrestricted. Plant extracts, at doses of 1500, 3000 and 5000 mg/kg body weight, respectively, were orally administered separately as suspensions in M9-DMSO to six male and six female mice. After dosing, each mouse was carefully observed at 2, 4, 24 and 48 h intervals for clinical signs, and less frequently twice daily for a continuous period of 14 days. The appearance of toxic symptoms such as behavioural changes, locomotion, convulsions and mortality, were observed and recorded. There is no law yet regulating animal research in Cameroon [13]. All animal-related experimental procedures were approved by the regional delegation of Livestock, Fisheries and Animal Industries (Nº075/16/L/RA/DREPIA). The present study was conducted in compliance with the Organization for Economic Cooperation and Development guidelines (420) for testing of chemicals [35].

2.7 Statistical Analysis

Results in each experiment are expressed as the mean values with their corresponding standard error of the mean determined using the Graphprism program 5.0 software. Data comparison was made using one way analysis of variance (ANOVA) followed by Tukey's test.

3. RESULTS

The ethanolic crude extracts from different parts of the tested plants showed an anthelmintic activity on macrofilariae of *Onchocerca ochengi* at 24, 48 and 72 h of incubation at 37ºC with LC_{50} values between 5 µg/mL to 300 µg/mL (Fig. 1).

Different parts of *D. microcarpum*, *G. senegalensis*, *T. emetica*, and *V. paradoxa* ethanolic extracts were tested for antifilarial activity using adults of *O. ochengi*. Ivermectin was the positive control. No mortality was observed during the experimental period of two weeks in the negative control M9-DMSO. Compared to ivermectin (LC_{50} values of 16.6 µg/mL) the ethanolic crude extract of stem bark and leaves of *D. microcarpum* exhibited the lower LC_{50} of 5 and 7.9 µg/mL ($p<0.001$) after 72 h on *O. ochengi* respectively (Fig. 1A).

The crude extract of stem barks and leaves of *D. microcarpum*, therefore, caused a higher mortality than the positive control ($p<0.001$). Fig. 1 shows most of the tested parts of *D. microcarpum* (Fig. 1A), *T. emetica* (Fig. 1B) and roots of *G. senegalensis* (Fig. 1D) exhibit macrofilaricidal activity at LC_{50} below that of the positive control ivermectin at the same exposure time (72 h).

Among the tested plants, crude extracts of the stem barks and leaves of *D. microcarpum* (LC_{50}= 5 µg/mL and 7.9 µg/mL respectively, Fig. 1A) are the most potent extracts against *O. ochengi*. The leaves and roots of *T. emetica* were the second most potent extracts (LC_{50} of 14.0 µg/mL, 12.5 µg/mL respectively, Fig. 1B). The extract of *G. senegalensis* showed lesser nematotoxicity (LC_{50}= 17.5 µg/mL and 12.5 µg/mL respectively, Fig. 1D). The leaves and stem barks of *V. paradoxa* induced the weakest mortality on *O. ochengi* after 72 h (LC_{50}= 50.0 µg/mL for both, Fig. 1C).

A phytochemical screening of the tested plant extracts revealed that flavonoids are globally the represented compounds in terms of quantity (Table 2). They are the main component in at least one part of each of the tested plant. From the plant extracts of leaves, stem barks and roots of *D. microcarpum* showed high amounts of the quantified phytochemical constituents (22 to 55 mg/100 g of dry plant product) represented by flavonoids. Nevertheless, the most important amount of compounds namely flavonoids is noticed on the leaves of *G. senegalensis* (174 mg). Elsewhere, saponins are the second leading compounds. They are highly represented in *V. paradoxa* (roots and stem barks), *T. emetic*

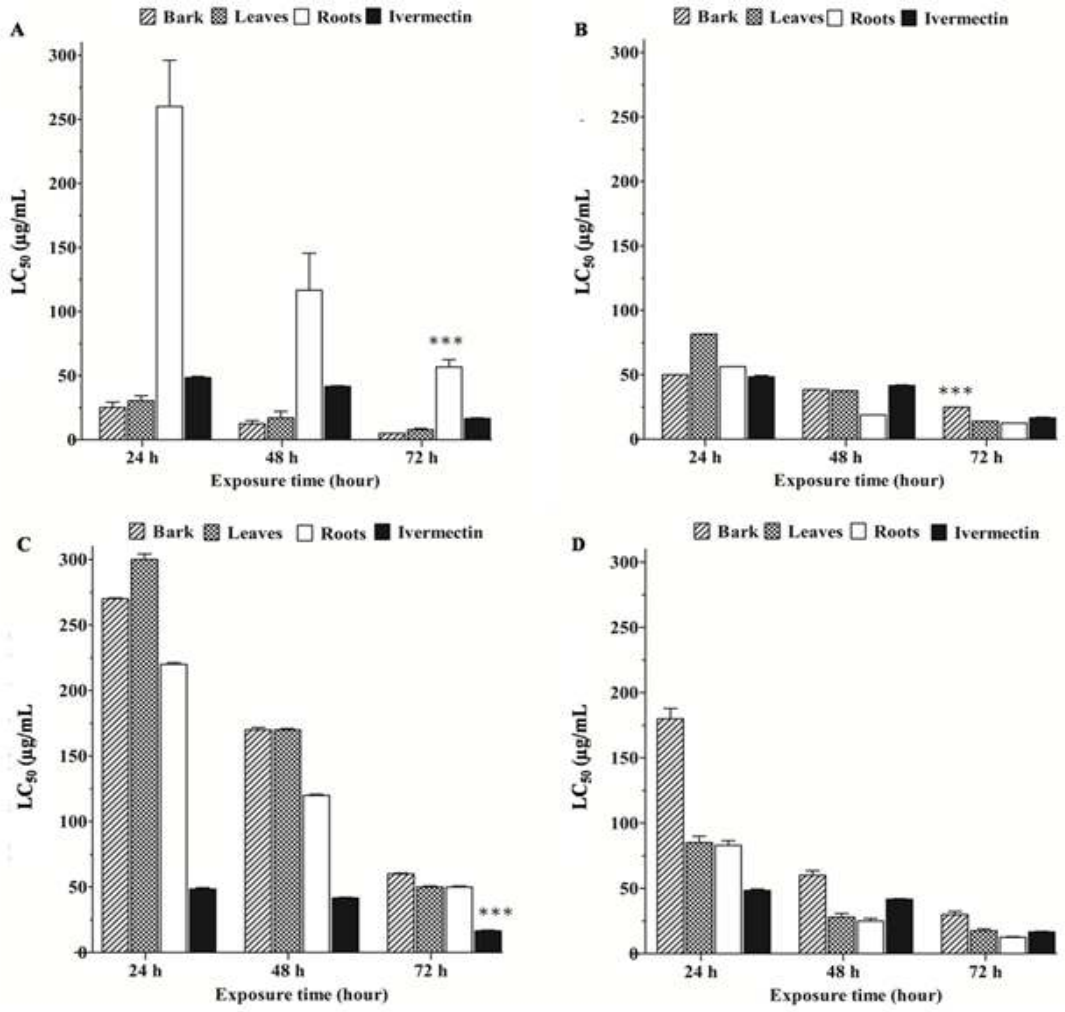

Fig. 1. Comparison of LC_{50} of *Onchocerca ochengi* exposed to different parts of the plants. The calculated LC_{50} for each time point was represented with standard error of the mean (SEM). (A) *Detarium microcarpum*; (B) *Trichilia emetica*; (C) *Vitellaria paradoxa*; (D). *Guiera senegalensis*
*(***: p<0.001)*

(leaves and roots) and *G. sengalesis*(roots and stem barks). Tannins are also represented with weak and very close amounts for all the screened plants (1-2.4 mg/100 g) except for roots of *G. senegalesis*. Phenolics are mostly represented as traces (≤0.65 mg/100 g) except for *D. microcarpum* leaves.

Acute toxicity results at doses 1500, 3000 and 5000 mg/kg of the ethanolic crude extracts of *D. microcapum, G. senegalensis* and *V. paradoxa* after 14 days showed no adverse effects and no mortality (Table 3).

The ethanolic crude extract of *T. emetica* showed 33.3% mortality at the dose of 1500 mg/kg and 100% mortality at doses up to 3000 mg/kg (Table 2). At the dose 1000 mg/kg no adverse reactions, no mortality were observed with the four plants tested (results not shown).

4. DISCUSSION

The increasing reliance on a single drug or compound risks the potential emergence of resistance. Plant extract is a cocktail of compounds which act synergically and can improve treatment effectiveness, reduce therapeutic duration and resistance. The present study was undertaken to assess the toxicity of the ethanolic crude extracts of *D. microcarpum*, *G. senegalensis*, *T. emetica*, and *V. paradoxa* on

Table 2. Quantity (mg) of phytochemical compounds (tannins, flavonoids, saponins and phenolics) for each 100 grams of dry leaves, stem barks and roots of *Detarium microcarpum*, *Vitellaria paradoxa*, *Trichilia emetica* and *Guiera senegalensis*

Plant	Part	Tannins (mg GAE)	Flavonoids (mg ER)	Saponins (mg GAE)	Phenolics (mg GAE)
D. microcarpum	Leaves	1.05 ± 0.05	55.00 ± 0.04	10.11 ± 0.58	3.67 ± 0.02
	Roots	1.58 ± 0.01	22.00 ± 0.00	14.43 ± 0.58	0.61 ± 0.03
	Stem barks	1.39 ± 0.01	48.00 ± 0.00	15.87 ± 1.57	0.54 ± 0.01
V. paradoxa	Leaves	2.368 ±0.00	48.00 ±0.00	23.07 ±0.05	0.65 ±0.02
	Roots	2.02 ± 0.13	12.00 ± 0.08	40.0 ± 5.50	0.45 ± 0.03
	Stem barks	1.49 ± 0.01	1.00 ± 0.00	37.47 ± 1.00	0.52 ± 0.02
T. emetica	Leaves	1.10 ± 0.02	9.00 ± 0.00	20.19 ± 0.58	0.44 ± 0.03
	Roots	1.03 ± 0.03	19.00 ± 0.01	18.75 ± 1.52	0.50 ± 0.01
	Stem barks	1.94 ± 0.01	17.00 ± 0.01	15.87 ± 0.58	0.35 ± 0.20
G. sengalesis	Leaves	2.18 ±0.01	174 ±0.02	2.07 ±1.53	0.55 ±0.01
	Roots	12.54 ± 0.05	1.02 ± 0.02	33.15 ± 0.58	0.63 ± 0.02
	Stem barks	1.31 ± 0.01	3.00 ± 0.00	40.35 ± 0.53	0.35 ±0.19

ER: rutin equivalent, GAE: Gallic acid equivalent

Table 3. Percentage (%) mortality of male and female mice 72 h after administration of crude extracts of *Detarium microcarpum*, *Guiera senegalensis*, *Trichilia emetica* and *Vitellaria paradoxa*

	Mortality rate of male and female mice (%)			
Crude extracts	**Control**	**1500 mg/kg**	**3000 mg/kg**	**5000 mg/kg**
D. microcarpum	-	0	0	0
T. emetica	-	33.3	100	100
V. paradoxa	-	0	0	0
G. senegalensis	-	0	0	0
Ivermectin	0	0	0	0
M9-DMSO	0	-	-	-

the bovine parasitic nematode *O. ochengi.* Our results demonstrate the sensitivity of the nematode to the different parts of the plant extracts.

Recent reports state that *D. microcarpum* is used in the treatment of constipation, dysentery, conjunctivitis, fever, itch, scabies, and wounds as well in the treatment of stomach aches, antimicrobial activity, anorexia and anemia [19-21]. *G. senegalensis* has been reported to have activity on protozoa such as *Plasmodium falciparum* and *Trypanosoma brucei bucei* [22,23]. Studies on *T. emetica* showed that this plant could heal malaria, cough, gastric ulcer, asthma, cirrhosis, intestinal worms, syphilis, infections of the skin and mouth infections. The stem barks were used as a purgative and leaves are used for healing of wounds [24-26,36]. *V. paradoxa* has also been demonstrated having activity against, schistosomiasis, headaches and treatment of sores, diarrhea and skin diseases [37,38]. In a separate study, a variety of bioactive components such as tannins (gallic acid, ellagic acid, galloylquinic acid and gentisic acid), flavonoids, alkaloids, phenols, tetranorditerpenoids, cis-2-oxokolavenic acid, copalic diterpen acid, coumarin, triterpen, leucoanthocyan, trichilin [39-48] have been isolated from *D. microcarpum*, *G. senegalensis*, *T. emetica* and *V. paradoxa*. Some of these phenolic compounds (gallic acid, ellagic acid and gentisic acid) obtained from other sources have shown strong activity against *O. ochengi* [11]. Remarkably, none of these selected plants except *T. emetica* have been tested on nematodes in general and on the bovine parasitic nematode *O. ochengi* in particular. However, some other plant extracts such as *Annona muricata, Azadirachta indica, Anogeissus leiocarpus, Coriandrum sativum, Tagetes minuta*, *Alpinia zerumbet, Tragia benthami, Piper umbellatum* [9,11,49-53], *Homalium africanum, Margaritaria discoidea, Alium sativum*, *Tagetes erecta, Craterispermum laurinum, Morinda lucida* [13,54-56], or *Acacia nilotica* [15] have shown, inhibitory properties against *Onchocerca* worms, gastrointestinal worms and glutathione

S-transferases. All those studies reported are not directly comparable to our results, due to differences in plant materials. Moreover, recent studies using plants mentioned above have also demonstrated that the majority of these plants contain almost the same bioactive compounds namely galloylated proanthocyanidins, polyphenols (gallic acid, ellagic acid, gentisic acid), tannins, flavonoids [11,15,45-48]. Those compounds have been shown to be active against *O. ochengi* and other nematodes. The activity observed on the filarial nematode *O. ochengi* might be attributed to the presence of bioactive compounds such as flavonoids and tannins in *D. microcarpum*, *G. senegalensis*, *T. emetica* and *V. paradoxa* and they might act synergically. Due to the presence of tannins in *D. microcarpum*, *G. senegalensis*, *T. emetica* and *V. paradoxa*, mortality observed might be explained by the activity of tannins. They react directly with surface proteins of the parasite (*O. ochengi*) causing a physiological dysfunction. The parasite mobility and the absorption of nutrients is obstructed leading to its death as observed by Katiki et al. [57]. It has been also demonstrated that tannins also interfere with the production of energy in helminth parasites by uncoupling the oxidative phosphorylation [54]. Another possible anthelmintic effect of tannins is that they can bind to glycoproteins on the cuticle of the parasite and can cause death [56,58]. The properties of this substance might explain possible modes of action of *D. microcarpum*, *G. senegalensis*, *T. emetica* and *V. paradoxa* because the majority of chemical families in these plants are tannins [19]. All these results confirm our findings with the selected four plant extracts; reinforce the existing knowledge and the regular use of the plants by traditional healers for the treatment of helminth infections.

The acute toxicity carried out with all the crude extracts, except with *T. emetica*, at a dose of 5000 mg/kg caused neither behavioural changes nor other signs of toxicity or even death in any of the mice tested during the 14 days observation period. Hence, the LD_{50} could not be determined. Any test substance showing an LD_{50} of 1000 mg/kg after oral administration can be considered safe [35]. This result indicates that the compounds under study, when given orally, could be considered relatively safe.

In summary, this work focused on the evaluation of the anthelmintic activity of ethanolic extracts from different parts of *Detarium microcarpum*, *Guiera senegalensis*, *Trichelia emetica* and *Vitellaria paradoxa* on nematodes *Onchocerca ochengi* the cattle parasites. It appears from our results that ethanolic extracts of all parts displayed nematicidal effects on adult worms of *Onchocerca ochengi*. We can say that *D. microcarpum*, *G. senegalensis*, *T. emetica* and *V. paradoxa* are important medicinal plants for their broad spectrum of uses.

5. CONCLUSION

These results allowed us to know that different parts of *D. microcarpus*, *T. emetica* and *G. senegalensis* more efficient than or as potent as ivermectin against male adults of *O. ochengi*. Acute toxicity results showed that at single-dose (1500, 3000 and 5000 mg/kg), there is no adverse effects of the ethanolic extract of *D. microcapum*, *G. senegalensis*, *V. paradoxa*. Nevertheless, additional work could be conducted using *T. emetic* fractions for getting more information about the safety of the active molecule(s). Those findings showed that *D. microcarpus, G. senegalensis* could be used in the traditional treatment of onchocerciasis. It would be interesting to extend this work and evaluate the synergistic effect of these three combined plants and a bioguided fractionation for the isolation of their active molecules.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Animal Ethical Committee of the Ngaoundere Regional Health Authority, Cameroon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zoure H, Noma M, Tekle A, Amazigo U, Diggle P, Giorgi E, Remme JH. 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 infected. Parasit Vectors. 2014;7:326.
DOI: 10.1186/1756-3305-7-326

2. Toé LD, Koala L, Burkett-Cadena ND, Traoré BM, Sanfo M, Kambiré SR, et al. Optimization of the esperanza window trap for the collection of the African onchocerciasis vector *Simulium damnosum* sensu lato. Acta Trop. 2014;137:39–43.
3. World Health Organization. Onchocerciasis. Geneva: World Health Organization; 2017. [Online] Available:http://www.who.int/mediacentre/factsheets/fs374/en/ (Accessed on 29 th April, 2017)
4. Katabarwa MN, Eyamba A, Nwane P, Enyong P, Kamgno J, Kueté T, et al. Fifteen years of annual mass treatment of onchocerciasis with ivermectin have not interrupted transmission in the west region of Cameroon. J Parasitol Res. 2013;2013:1–12.
5. Kamga G-R, Dissak-Delon FN, Nana-Djeunga HC, Biholong BD, Mbigha-Ghogomu S, Souopgui J, et al. Still mesoendemic onchocerciasis in two Cameroonian community-directed treatment with ivermectin projects despite more than 15 years of mass treatment. Parasit Vectors. 2016; 9.
DOI: 10.1186/s13071-016-1868-8
6. Noma M, Zouré HG, Tekle AH, Enyong PA, Nwoke BE, Remme JH. The geographic distribution of onchocerciasis in the 20 participating countries of the African programme for onchocerciasis control: (1) priority areas for ivermectin treatment. Parasit Vectors. 2014; 7:325.
7. Kuesel AC. Research for new drugs for elimination of onchocerciasis in Africa. Int J Parasitol Drugs Drug Resist. 2016;6(3): 272–86.
8. Osei-Atweneboana MY, Awadzi K, Attah SK, Boakye DA, Gyapong JO, Prichard RK. Phenotypic evidence of emerging ivermectin resistance in *onchocerca volvulus*. PLoS Negl Trop Dis. 2011;5(3). Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3066159/
9. Cho-Ngwa F, Monya E, Azantsa BK, Manfo FPT, Babiaka SB, Mbah JA, et al. Filaricidal activities on *Onchocerca ochengi* and Loa loa, toxicity and phytochemical screening of extracts of *Tragia benthami* and *Piper umbellatum*. BMC Complement Altern Med. 2016;16(1). Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5004253/
10. Metuge JA, Nyongbela KD, Mbah JA, Samje M, Fotso G, Babiaka SB, Cho-Ngwa F. Anti-Onchocerca activity and phytochemical analysis of an essential oil from *Cyperus articulatus* L. BMC Complement Altern Med. 2014;14:223. DOI: 10.1186/1472-6882-14-223
11. Ndjonka D, Abladam ED, Djafsia B, Ajonina-Ekoti I, Achukwi MD, Liebau E. Anthelmintic activity of phenolic acids from the axlewood tree *Anogeissus leiocarpus* on the filarial nematode *Onchocerca ochengi* and drug-resistant strains of the free-living nematode *Caenorhabditis elegans*. J Helminthol. 2014;88(04):481–8.
12. Metuge JA, Babiaka SB, Mbah JA, Ntie-Kang F, Ayimele GA, Cho-Ngwa F. Anti-onchocerca Metabolites from *Cyperus articulatus*: Isolation, *In Vitro* Activity and In Silico. 'Drug-Likeness'. *Nat Prod Bioprospect*. 2014;4:243-249.
13. Samje M, Metuge J, Mbah J, Nguesson B, Cho-ngwa F. *In vitro* anti- *Onchocerca ochengi* activities of extracts and chromatographic fractions of *Craterispermum laurinum* and *Morinda lucida*. BMC Complement Altern Med. 2014;14:325.
DOI: 10.1186/1472-6882-14-325
14. Doyle SR, Armoo S, Renz A, Taylor MJ, Osei-Atweneboana MY, Grant WN. Discrimination between *Onchocerca volvulus* and *O. ochengi* filarial larvae in *Simulium damnosum* (s.l.) and their distribution throughout central Ghana using a versatile high-resolution speciation assay. Parasit Vectors. 2016; 9. Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5057476/
15. Dikti VJ, Kalmobe J, Djafsia B, Schmidt TJ, Liebau E, Ndjonka D. Anti-onchocerca and Anti-caenorhabditis activity of a hydro-alcoholic extract from the fruits of *Acacia nilotica* and Some proanthocyanidin derivatives. Molecules. 2017;22:748. DOI:10.3390/molecules22050748
16. Ndjonka D, Ajonina-Ekoti I, Djafsia B, Luersen K, Abladam E, Liebau E. A*nogeissus leiocarpus* extract on the parasite nematode *Onchocerca ochengi* and on drug resistant mutant strains of the free-living nematode *Caenorhabditis elegans*. Vet Parasitol. 2012;190:136–42.

17. Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achukwi D, Nukenine EN, et al. *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. J Helminthol. 2011;85(03):304–12.
18. Burkill HM. Useful plants of west tropical Africa. Families J - L. 2nd Ed., Whitefriars Press, London. Kew, United Kingdom, Royal Botanic Gardens. 1997;3:101-05.
19. Kouyaté AM, Lamien N. *Detarium microcarpum*, sweet detar." Conservation and Sustainable Use of Genetic Resources of Priority Food Tree Species in sub-Saharan Africa. *Bioversity International* 4; 2011, 1-8 Accessed November 24, 2012.
20. Teddy E, Chimezie A, Bola M. Heavy metals content in the stem bark of *Detarium microcarpum* determined by atomic absorption spectrophotometer. Afri J Biotechnol. 2013;12(11):1236-38.
21. Yerbanga RS, Lucantoni L, Ouédraogo RK, Da DF, Yao FA, Yaméogo KB, et al. Transmission blocking activity of *Azadirachta indica* and *Guiera senegalensis* extracts on the sporogonic development of *Plasmodium falciparum* field isolates in *Anopheles coluzzii* mosquitoes. Parasit Vectors. 2014;7:185.
22. Traore MS, Diane S, Diallo MST, Balde ES, Balde MA, Camara A, et al. *In vitro* antiprotozoal and cytotoxic activity of ethnopharmacologically selected guinean plants. Planta Med. 2014;80(15):1340–4.
23. Biu AA, Buratai LB, Onyedim PN, Hambali IU, Ngulde SI, Zakariah M, et al. Phytochemistry, toxicity and in vitro antitrypanosomal efficacy of crude aqueous extract of *Guiera senegalensis* stem bark. Bangl J Vet Med. 2016;14(1):93–7.
24. Tchacondo T, Karou SD, Agban A, Bako M, Batawila K, Bawa ML, et al. Medicinal plants use in central Togo (Africa) with an emphasis on the timing. Pharmacognosy Res. 2012;4(2):92–103.
25. Moyo P, Botha ME, Nondaba S, Niemand J, Maharaj VJ, Eloff JN, et al. *In vitro* inhibition of *Plasmodium falciparum* early and late stage gametocyte viability by extracts from eight traditionally used South African plant species. J Ethnopharmacol. 2016;185:235–42.
26. Bobach C, Schurwanz J, Franke K, Denkert A, Sung TV, Kuster R, et al. Multiple readout assay for hormonal (androgenic and antiandrogenic) and cytotoxic activity of plant and fungal extracts based on differential prostate cancer cell line behavior. J Ethnopharmacol. 2014;155(1):721–30.
27. Diallo D, Paulsen BS, Liljeback THA, Michaelsen TE. The malian medicinal plant *Trichilia emetica*, studies on polysaccharides with complement fixing ability. J Ethnopharmacol. 2003;84:279–87.
28. Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SMC, et al. Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pacific J Trop Med. 2014;7:S442–7.
29. Jansen O, Angenot L, Tits M, Nicolas JP, De Mol P, Nikiéma J-B, et al. Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. *J Ethnopharmacol*. 2010; 130(1):143–50.
30. Fodouop SPC, Tala SD, Keilah LP, Kodjio N, Yemele MD, kamdje Nwabo AH, et al. Effects of *Vitellaria paradoxa* (C.F. Gaertn.) aqueous leaf extract administration on *Salmonella typhimurium*-infected rats. BMC Complement Altern Med. 2017;17.
31. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of anaqueous extract of *Coleus aromaticus*. Food Chem. 2006;97:109-14.
32. Wolfe K, WU X, LIU RH: Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry. 2003;51(3):609–614.
33. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci. 2001;8:203-208.
34. Borsboom GJ, Boatin BA, Nagelkerke NJ, Agoua H, Akpoboua KL, Alley EW, Bissan Y, Renz A, Yameogo L, Remme JH, Habbema JD. Impact of Ivermectin on onchocerciasis transmission: Assessing the empirical evidence that repeated Ivermectin mass treatements may lead to elimination/eradication in West-Africa. Filaria J. 2003;2:8.
35. Organization for Economic Co-operation and Development (OECD). Guidelines for the Testing of Chemicals. Paris. 2001; Monograph No 420.
36. Komane BM, Olivier EI, Viljoen AM. *Trichilia emetica* (Meliaceae) – A review of

traditional uses, biological activities and phytochemistry. Phytochemistry Letters. 2011;4(1):1–9.

37. Abubakar K. Evaluation of the antidiarrhoeal effect of *Vitellaria paraoxa* Gaertn F (Sapotaceae) stem bark extract. Advances in Life Science and Technology. 2013;15.
ISSN 2224-7181 (Paper) ISSN 2225-062X.
38. Bah S, Diallo D, Dembélé S, Paulsen BS. Ethnopharmacological survey of plants used for the treatment of schistosomiasis in Niono District, Mali. *J Ethnopharmacol*. 2006;105(3):387–99.
39. Cisse M, Dieme O, Diop N, Dornier M, Ndiaye A, Sock O. Le ditax (*Guiera senegalensis* J. F. Gmel.): Principales caractéristiques et utilisations au Sénégal. Fruits. 2010;65(5):293-306.
40. Lamien-Meda A, Lamien CE, Compaoré MM, Meda RN, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules. 2008;13(3):581-94.
41. Cavin AL, Hay AE, Marston A, Stoeckli-Evans H, Scopelliti R, Diallo D, Hostettmann K. Bioactive diterpenes from the fruits of *Detarium microcarpum*. J Nat Prod. 2006;69(5):768-73.
42. Fiot J, Sanon S, Azas N, Mahiou V, Jansen O, Angenot L, Balansard G, Ollivier E**.** Phytochemical and pharmacological study of roots and leaves of *Guiera senegalensis* J.F. Gmel (Combretaceae). J Ethnopharmacol. 2006;106(2):173-78.
43. Lamien CE, Meda A, Couacy-Hymann E, Ouedraogo AG, Nacoulma OG. The phytochemical composition and *in vitro* antiviral activity of decoctions from galls of *Guiera senegalensis* J.F. Gmel. (Combretaceae) and their relative non-toxicity for chickens. Onderstepoort J Vet Res. 2005;72(2):111-18.
44. Germanò MP, D'Angelo V, Biasini T, Sanogo R, De Pasquale R, Catania S. Evaluation of the antioxidant properties and bioavailability of free and bound phenolic acids from *Trichilia emetica* Vahl. J Ethnopharmacol. 2006;105(3):368-73.
45. Ramsay A, Williams AR, Thamsborg SM, Mueller-Harvey I. Galloylated proanthocyanidins from shea (*Vitellaria paradoxa*) meal have potent anthelmintic activity against *Ascaris suum*. Phytochemistry. 2016;122:146-53.
46. Eyong KO, Foyet HS, Baïrys G, Ngosong Folefoc G, Acha Asongalem E, Lagojda A, Lamshöft M. A new ursane triterpenoic acid and other potential anti-inflammatory and anti-arthritic constituents from EtOAc extracts of *Vitellaria paradoxa* stem bark. J Ethnopharmacol. 2015;174:277-86.
47. Zhang J, Kurita M, Shinozaki T, Ukiya M, Yasukawa K, Shimizu N, Tokuda H, Masters ET, Akihisa M, Akihisa T. Triterpene glycosides and other polar constituents of shea (*Vitellaria paradoxa*) kernels and their bioactivities. Phytochemistry. 2014;108:157-70.
48. Baatile MK, Eugene IO, Alvaro MV. *Trichilia emetica* (Meliaceae) A review of traditional uses, biological activities and phytochemistry. Phytochemistry Lett. 2011;4:1–9.
49. Ferreira LE, Castro PM, Chagas AC, França SC, Beleboni RO. *In vitro* anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. Exp Parasitol. 2013;134:327–32.
50. Quelemes PV, Perfeito MLG, Guimarães MA, dos Santos RC, Lima DF, Nascimento C, et al. Effect of neem (*Azadirachta indica* A. Juss) leaf extract on resistant *Staphylococcus aureus* biofilm formation and *Schistosoma mansoni* worms. J Ethnopharmacol. 2015;175:287–94.
51. Mukherjee N, Saini P, Mukherjee S, Roy P, Gayen P, Sinha Babu SP. Ethanolic extract of *Azadirachta indica* (A. Juss.) causing apoptosis by ROS upregulation in *Dirofilaria immitis* microfilaria. Research in Veterinary Science. 2014;97(2):309–17.
52. Soro D, Koné WM, Bonfoh B, Dro B, Toily KB, Kamanzi K. *In vivo* anthelmintic activity of *Anogeissus leiocarpus* Guill & Perr (Combretaceae) against nematodes in naturally infected sheep. Parasitol Res. 2013;112(7):2681–8.
53. Macedo ITF, Oliveira LMB de, Camurça-Vasconcelos ALF, Ribeiro WLC, Santos JML dos, Morais SM de, et al. *In vitro* effects of *Coriandrum sativum*, *Tagetes minuta*, *Alpinia zerumbet* and *Lantana camara* essential oils on *Haemonchus contortus*. Revista Brasileira de Parasitologia Veterinária. 2013;22(4):463–9.
54. Cho-Ngwa F, Abongwa M, Ngemenya MN, Nyongbela KD. Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca*

ochengi. BMC Complement Altern Med. 2010;10:62.
Available:http://dx.doi.org/10.1186/1472-6882.

55. Palacios Landín J, Mendoza de Gives P, Salinas Sánchez DO, López Arellano ME, Liébano Hernández E, Hernández Velázquez VM, et al. *In vitro* and *in vivo* nematocidal activity of *Allium sativum* and *Tagetes erecta* extracts against *Haemonchus contortus*. Turkish Journal of Parasitology. 2016;39(4):260–4.
56. Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother Res. 2000;14:630–34.
57. Katiki LM, Ferreira JFS, Gonzalez JM, Zajac AM, Lindsay DS, Chagas ACS, Amarante AFT. Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. Vet Parasitol; 2012.
DOI: org/10.1016/j.vetpar.2012.09.030
58. Thompson DP, Geary TG. The structure and function of helminth surfaces, in: Marr JJ. (Eds.), Biochemistry and molecular biology of parasites, 1st ed. Academic Press, New York. 1995;203-32.

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