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Original Research Article

Phytochemical Analysis, Acute Toxicity and *In vitro* Activity of Ethanolic Leaves Extract of *Waltheria indica* (Sterculiaceae) on *Onchocerca ochengi* and *Caenorhabditis elegans*

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Abstract: Onchocerciasis is responsible of a considerable burden of diseases, mainly because of its symptoms: visual impairment, blindness, disfiguring skin lesions, and severe itching, which are the results of the death of microfilariae. Since these infections are predominantly widespread in the poor and developing nations Ivermectin, the current drug of choice, is only microfilaricidal and suboptimal response to it, is increasingly being reported. Adult males of Onchocerca ochengi were incubated in RPMI-1640 medium supplemented of penicillin/streptomicin and plant extract or drugs. Caenorhabditis elegans were incubated at 20°C in NGM-Agar seeded and E. coli OP50 supplemented of plant extract or drugs. Worm mortality was determined biochemically by MTT/formazan colorimetry after 24, 48 and 72 h. Ivermectin and levamisole were used as positive controle. DMSO was the negative control. Acute toxicity on mice (Mus musculus) and phytochemical compounds were also determined. ELE of W. indica was lethal against O. ochengi and C. elegans. O. ocheng was the most sensitive to the extract with LC50. Values between 4.03 ± 0.51 µg/ml and 27.46 ± 0.08 µg/ml. The determination of secondary metabolites has revealed the presence of tannins, phenolic acids, saponins and flavonoids. Saponins (12.39 ± 1.00 mg/g) were the most quantified compound followed by tannins (0.96 ± 0.07 mg GAE/ 100g), phenolic acid (0.51 ± 0.07 mg/g GAE) and flavonoids (0.04 ± 0.07 mg of rutin/ 100g). The acute toxicity results showed that the LD50 is greater than 5000 mg/kg. The data indicate that the plant extract could be used to treat nematode infections even in cases of drug resistance towards established anthelmintic drugs.

Keywords: Onchocerca Ochengi, Caenorhabditis Elegans, Waltheria Indica, Ivermectin, Levamisole.

INTRODUCTION

Helminths are macroscopic worms causing a wide variety of diseases globally called helminthiases. There are three different kinds of helminths: Platyhelminths (flat worms), Nemathelminths (non-segmented roundworms) and Annelida (segmented round worms) [1]. Those infecting human belong to the first two groups. Among Nemathelminths, there are *O. volvulus* and *O. ochengi* which cause Human onchocerciasis and cattle onchocerciasis respectively. Human Onchocerciasis or river blindness is an infectious parasitic disease transmitted by the blood-feeding blackflies of the genus *Simulium damnosum* [2]. Female worm can produce millions of embryos each day during her lifetime (10-15 years) [3]. Pathologically, the disease is associated with extensive and disfiguring skin changes, musculoskeletal complaints, weight loss and changes in the immune system [4]. In addition to its severe pathological effect, it causes worse socio-economic problems and life-long human suffering [5]. It is one of the ten tropical neglected diseases [6] and more than 90% of all human onchocerciasis cases are found in sub-Saharah Africa, the rest occur in isolated locations in Yemen and Central and South America [7]. About 37

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million persons are infected presently with *O. volvulus*, and approximately, 270,000 are blind and 500,000 visually impaired [8, 9].

Several strategies have been used to control ochocerciasis over the past years aimed at targeting the vector and the parasitic stage of O. volvulus in the human host. [8; 9]. In spite of the successes reported in reducing the disease burden, total elimination of onchocerciasis has not been achieved and remains always a major public health problem. Nowaday, ivermectin is the major applied drug approved for treatment of onchocerciasis and has been the cornerstone of the Onchocerciasis Control Program (OCP) and later the African Program for Onchocerciasis Control (APOC) [10]. Although this drug has been shown to significantly reduce transmission of the disease, its filaricidal effect is limited only on the juvenile form of the parasite [11, 12]. Also, the emergence of ivermectin resistance in parasitic nematodes of veterinary importance [13] raises serious concerns that this may extend to the human *O. volvulus*. Besides, suboptimal response to ivermectin has also been reported in different studies [13, 14]. Hence, there is a need to find medicinal plants with microfilaricidal and macrofilacidal properties for new compounds against onchocerciasis. In very remote areas where it's very difficult to access modern medicine services, the inhabitants mainly resort to herbal medicine for their health care needs. Thus, traditional medicine has gained popularity as a source of primary health care worldwide because of its affordability and social acceptance [15]. Many diseases in the rural areas can be prevented or treated through simple means by traditional healers [15]. Traditional medicine can also be used for prevention of certain diseases through measures against vectors and parasites [15]. In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care and Herbal medicines are the most lucrative form of traditional generating billions of dollars of revenue [16]. Owing to the lack of a laboratory host for the human parasite O. volvulus, model organisms are often needed. Therefore, the bovine parasite O. ochengi and the free-living nematode Caenorhabditis elegans have been used. O. ochengi and C. elegans are evolutionary closely related and are transmitted to their human and bovine host by the same blackfly vector Simulium damnosum. Rencently, a number of studies reported the promising results about plant extracts against Onchocerca [17-20]. Thus in this study, W. indica was selected based on their wide usage by traditional healers, for the treatment of human and livestock parasites in the northern part of Cameroon. Waltheria. indica L. belongs to the family Sterculiaceae. It is an erect perennial shrublet up to±500 mm high, stalked leaves with margins shallowly and irregularly toothed [21]. Its flowers are yellow and occur in clusters. Globally, its distribution and habitat are mostly in subtropical and tropical zones, in scrub forests, inundated savannas, riverbanks, sandy or clay soils, and in disturbed or impoverished soils [22]. W. indica, is commonly called sleepy morning, monkey bush, velvet leaf, marsh-mallow, boater bush, guimauve, "hankufah" in Hausa, "kafafi" (local name) in Fulfulde, "matum kevel" in Wolof [23-25]. Several previous studies have revealed the properties of W. indica. Thus, roots extracts have been reported to be highly active against Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and trypanosome parasites [26, 27]. Ethanolic extracts of stems, roots, and leaves of W. indica have been reported to possess potent activity against a variety of gram-negative strains, with the largest zone of inhibition of 15 mm against Citrobacter freundii [28]. This work aims is to evaluate the activity of ELE of W. indica against O. ochengi and C. elegans.

MATERIALS AND METHODS

Plant Materials and Preparation of Extract

Leaves W. indica were collected in June 2013 at dang (latitude 07°24'9.949"N, longitude 13°32'870" E and altitude 199 m above sea level). Botanical's plant identification was done at the national herbarium of Cameroon in Yaounde, where voucher specimens were kept and registered under number 8994/SRFK. The extraction was performed using the modified method described by Ojezele *et al.,*[29]. Briefly, leaves of W. indica were harvested early in the morning, washed and dried in the shade at room temperature. The dry part was ground and sieved on a 0.5 mm mesh screen. Hundred (100) mg of the powder was weighed and macerated for 48 hours in 100 ml of ethanol (70%). The mixture was filtered over filter papers No. 413 (VWR International, Darmstadt, Germany). The filtrate was then concentrated under reduced pressure by rotary evaporation (BUCHI Rotavapor R-200, Switzerland) at 40°C. Residual solvent was removed by drying in a sweating-room at 35°C and the extract was weighed and stored at + 4°C. For further investigation, the leaves extract of W. indica was dissolved in dimethyl sulfoxide (DMSO) and RPMI (Roosevelt Memorial Park) to a final concentration of 100 mg/ml, filtered and aliquoted. Afterward its activity was assessed on C. elegans strains and O. ochengi.

Preparation of Ivermectin, Levamisole

The positive control was made of ivermectin and levamisole. They were dissolved in 10% dimethyl sulfoxide (DMSO) and diluted with RPMI and distilled water. The final concentrations of both drugs for RPMI-stock solutions was 2 mg/ml. DMSO was used as negative control. The maximal concentration of DMSO used was lower 1%.

Extraction and Isolation of Parasites *Onchocerca ochengi*

The isolation O. ochengi adult worms was done according to the modified process used by Cho-Ngwa *et al.,*[30]. Portions of umbilical cattle skin with palpable nodules bought from local slaughterhouse were washed, drained and sterilized with 70% ethanol. Nodules were isolated from the skin, using a scalpel, by making a slight tear on the inner face of the skin. The isolated nodules were placed in a solution of PBS (Phosphate Buffered Saline) for manual dissection. The viability of worms retained for the assay was ascertained using inverted microscope (Euromex, Holland) examination and damaged worms were discarded. Following the modified protocol of Borsboom *et al.,*[31], adult worms were then incubated at 37°C under 5% CO2, in 96-wells microtitre plates (one male per well in 100 µl medium) in RPMI 1640 containing different concentrations of ELE of W. indica or drug. The mortality was determined after 24, 48 and 72 h.

In vitro* Assay on *Onchocerca ochengi

The assay was process following technical established by Ndjonka *et al.,*[31]. Briefly, after isolation, damaged worms and worms from putrefied nodules were discarded. The viability of worms retained for the assay was ascertained by microscopic examination of adult worm motility (Euromex, Holland) dissecting microscope. The normal worms were carried under the hood and washed 3 times in the plates containing sterile PBS and then twice in the RPMI (Roosevelt Memorial Park Medium-1640, SIGMA, USA) which is the culture medium. Worms were incubated in different concentrations of the plant extract or drug prepared solutions in RPMI 1640 supplemented with penicillin/streptomycin (100 U/100µg/ml). The assay was conducted in 96-well microtitre plates (one macrofilariae in 100 µl per well) at 37°C under an atmosphere of 5% CO2 in air for 3 days. The mortality was determined after 24 h, 48 h and 72 h.

Culture of *Caenorhabditis elegans*

The different strains of C. elegans (wild type N2 Bristol) and the mutant strains CB211 lev-1 (e211) IV, VC722 glc-2 (ok1047) I) used in this work were provides by Prof. E. Liebau, Zoology Institute of the University of Münster, Germany. Cultures were realized as described by Ndjonka *et al.,*[32]: worms were grown at 20°C under standard monoxenic conditions on NGM-agar (Nematode Growth Medium: 2.5 g peptone from casein, 3 g NaCl, 17 g agar, 0.5% cholesterin, 1 mM CaCl2, 1 mM MgSO4, 25 mM KH2PO4/K2HPO4 in 1 l of water) in Petri dishes seeded with Escherichia coli OP 50 to serve as food source. Petri dishes of 85 mm with gravid adult worms were selected for synchronization [33].

Synchronization of *Caenorhabditis elegans* and Assays

The content of the plate was rinsed while stirring for 5 to 6 minutes. The supernatant was recovered in eppendorf tubes then centrifuged at 7500 rpm for 1 min at 4°C. The supernatant was treated for 6 min with a mixture of distilled water, alkaline sodium hypochlorite (12% NaOCl) and sodium hydroxide (0.5 M NaOH). Then, the mixture was centrifuged at 8500 rpm for 1 min at 4°C. The supernatant was discarded and the pellet containing only the viable eggs was rinsed 3 times in 1mL of M9 buffer and whole was centrifuged at 4000 rpm for 1 min at 4°C. The pellet was kept in 200 µl of M9 during the last rinse and then divided into two Petri dishes (100 µl per dish) containing NGM seeded with E. coli OP50. After 48 hours of incubation at 20°C, the young adults or L4 larvae of the same age were used to test plant's toxicity [33, 34]. The synchronized worms (ten young L4 adults per Petri dish) were transferred and incubated with different concentrations of plant extracts or drug on NGM-Agar seeded with dried E. coli OP50 (80 µl). Each small petri dish (35 mm in diameter) received 3 ml of hot NGM-Agar (about 40°C), to which the volume of extract's stock solution corresponding to each concentration was mixed. Negative control plates did not contain plant extracts or drug. Positive controls were performed with levamisole and ivermectin. Three trials were conducted for each concentration and the worms were incubated at 20°C. The mortality was determined after 24 h and 48 h and 72 h [35].

Determination of Worm Mortality

Worms were removed from their wells, then washed with PBS (phosphate-buffered saline) and subjected to the MTT colorimetric assay in order to recognize the dead or living worms. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) is a pale-yellow compound which is reduced to a dark blue product, formazan by the living cells of the worms. Single intact worms are placed in each well of a 48-well plate (Falcon, UK) containing 505 µL of a solution consisting of 5 µL MTT (0.5 mg/mL) and 5 µL of RPMI, then incubated at 37°C and observed after 30 min under a binocular microscope. Thus, after incubation, when the worms were alive, they were colored blue because the MTT was reduced to formazan. Dead worms do not reduce MTT to formazan but simply take on the yellow color of MTT. All the MTT assays were done in the dark since the MTT reagent is sensitive to light [36].

Phytochemical Analysis

The phenolic acids content was performed using the FolinCiocalteu modified method of Folin and Denis. [37], which consists in an evaluation of gallic acid content in a serie of increasing concentrations of its aqueous solution. A titration curve of gallic acid at a

wave length of 765 nm was then drawn from the evaluated absorbance. Succinctly, to 50 µl of the sample-solution, 200 µl of Na2CO3 and 250 µl of a 1/10 (v/v) of FC reagent were successively added. The obtained solution was agitated manually for one minute and incubated in dark at 40°C for 30 minutes. The absorbance was determined at 765 nm using a spectrophotometer (UVbiowave Cambridge, England). The phenolic acids content was calculated from the standard curve of gallic acid titration's equation (linear regression equation). Tannins and phenolic acids contents were expressed as equivalent of gallic acid per gram of dry plant material (mg GAE/100g). The tannins quantification was conducted according to the modified method of of Pradeepa *et al.,*[38]. For tannins determination 200 µL of a solution of the sample were mixed with 35% (w/v) of Na2CO3 and 100 µL of Folin-Ciocalteu (FC) reagent were added. The whole was mixed by vortexing for one minute and incubated five minute at room temperature (25 to 30°C). The absorbance was then read at 640 nm. The flavonoids were quantified according to the modified method described by Wolfe *et al.,*[39]. Briefly, to 0.1 g of the extract, 2 ml of the extract solvent made of 140:50:10 methanol-distilled water-acetic acid were added. The obtained solution was filtered using a wattman paper and an equal volume of extraction solvent was added. 250 µl of the filtrate was transfered to a 5 ml tube and completed to 5 ml with distilled water which constitutes the analysis solution (A solution). For titration, to 5 ml of the A solution, 200 µl of distilled water and 500 µl of aluminium chlorite solution (133 mg of AlCl3 and 400 mg of sodium acetate in 100 ml distilled water) were added and the solution mixed by vortexing. A rutin titration curve was set at the absorbance of 430 nm and the flavonoids amount was expressed as mg of rutin/100g of dry material. The saponins content was determined following the modified method described by Jamuna Senguttuvan, (2014). 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 foam in cm after 5 to 10 sec) + 0.008] / weight of sample in gram).

Acute Toxicity Test

The oral acute toxicity of ELE of W. indica was performed using the method of Adedapo *et al.,*[40]. Twenty-four healthy adult male and female albino mice weighing 60-120 g and of 10 weeks old were used. The assay was done according to the OECD guideline [41]. The animals were obtained from LANAVET (National Veterinary Laboratory, Garoua, Cameroon). They were separated into 4 groups of 6 animals (3 males and 3 females) and identified by color tail marks. They have been deprived of food for 12 h and received orally different doses of the extract (760, 1520, 5000 mg/kg) using a feeding needle corresponding to groups A, B, C and a control group which received distilled water. All the animals were then allowed free access to food and water and observed at 2, 4, 24 and 48 h for any clinical signs of toxicity such as behavioral changes, locomotion, convulsions, lost appetite and sneezing. The toxic clinical manifestations were recorded at the indicated time points, and thereafter twice daily for a continuous period of 14 days. The numbers of deaths within this period were recorded.

Statistical Analysis

The Graphprism program 5.0 software was used to set out the curves. The LC50 values were determined by Probit Analysis method using SPSS 16.0 Turkey's test at P=0.05 was applied for mean separation. The LC50 values were expressed as mean±standard deviation (mean of 3 replicates).

RESULTS

Table 1: LC_{50} values of the ELE of *Waltheria indica* and reference drugs (ivermectin and levamisole) tested against *O. ochengi* and *C. elegans* (wild type and drug-resistant strains) after 24, 48 and 72 h of exposure

LC50 (µg/mL) (95%FL) Khi-carré									
	24h			48h			72h		
	Leaves	**Ivermectin**	**Levamisol**	**Leaves**	**Ivermectin**	**Levamisole**	**Leaves**	**Ivermectine**	**Levamisole**
O. ochengi	27.46 ± 0.08^{abcdA} (17.22-38.73) 98.85***	0.18 ± 0.06^{aB} (0.15-1.44) 137.25***	nd	11.02 ± 0.12^{abcdA} (5.37-16.20) 79.39***	0.10 ± 0.07^{aB} ns (0.05-0.13) 45.97***	nd	4.03 ± 0.51^{abcA} (1.46-6.93) 28.10***	0.07 ± 0.04^{aB} (0.04-0.84) 47.89***	nd
C. elegans WT	17.59 ± 0.12^{baA} (12.84-22.23) 16.75 ns	2.75 ± 1.42^{bB} ns (1.54-3.56) 15.25 ns	5.82 ± 0.31^{bC} (4.97-6.51) 17.97 ns	12.72 ± 0.16^{bacdA} (5.96-19.12) 37.81***	2.45 ± 0.41^{bBC} (0.95-3.59) 20.25*	3.94 ± 0.64^{bCB} (2.76-4.73) 19.21 ns	8.58 ± 0.20^{bcdABC} (2.25-14.96) 45.58***	2.23 ± 1.35^{bBAC} ns (1.22-3.88) 43.54***	3.56 ± 1.16^{bCAB} ns (2.74-4.74) 38.80***
C. elegans CB211	20.99 ± 0.11^{caA} ns (10.54-30.68) 37.47***	nd	>100	18.18 ± 0.13^{cabdC} ns (8.25-27.15) 66.79***	nd	>100	11.54 ± 47.47^{cabdA} ns (4.59-18.07) 47.47***	nd	>100
C. elegans VC722	40.99 ± 0.11^{daA} (31.73-49.67) 31.73***	>100	nd	25.94 ± 0.11^{dabcD} ns (14.19-36.45) 49.09***	>100	nd	19.31 ± 0.13^{dbcA} ns (9.32-28.17) 70.41***	>100	nd

The LC_{50} values are expressed as mean ± standard deviation (SD) of mean of 3 replicates. Small letters compare means in a column and capital letters means in a row. Different letters indicate significant difference at **p = 0.05**. **nd:** not determinated; **FL:** Fiducial Limit; **ns:** not significant; ***p = 0. 05**; ****p = 0.001.**

The LC_{50} values of the ELE of *W. indica*, ivermectin and levamisole on the two nematodes obtained after 24 h, 48 h and 72 h are presented in Table 1. These results show that the LC_{50} values of the plant extract range from 4.03 ± 0.51 µg/ml to 40.99 ± 0.11 µg/ml and those of the drugs from 0.07 ± 0.04 µg/ml to 5.82 ± 0. 31 µg/ml. Plant extract and drugs were most lethal for *O. ochengi.* Generally, the LC_{50} values obtained show that ivermectin has the lowest LC_{50} values (0.07 ± 0.04 µg/ml, 0.10 ± 0.07 µg/ml, 0.18 ± 0.06 µg/ml). Between the LC_{50} values of the ELE of *W. indica* and those of drugs tested on Wild-type of *C. elegans*, the difference is not significant after 72 h incubation.

Phytochimical analysis

Table 2: Phytochimical compounds (mg) determined in ELE of *Waltheria indica*. For each 100 grams of the ELE of *Waltheria indica*. GAE: Gallic Acid Equivalent. Figures are expressed as mean ± SD

Waltheria indica	Tannins (mg/g GAE)	Flavonoids (mg rutin/100g)	Saponins (mg/g GAE)	Phenolic acids (mg/g GAE)
Leaves (100g)	0.96 ± 0.07	0.0 4 ± 0.07	12.39 ± 1.00	0.51 ± 0.07

Results of the quantitative phytochemical analysis (mg) of tannins, flavonoids, saponins and phenolic acids present in ELE of *W. indica* revealed variable values. Saponins had the lowest (12.39 ± 1.00 mg / g GAE), followed by tannins (0.96 ± 0.07 mg / g GAE), phenolic acids (0.51 ± 0.07 mg / g GAE) and flavonoids (0.0 4 ± 0.07 mg rutin / 100g).

Acute toxicity study

Acute toxicity of the ELE of *W. indica* revealed some signs of toxicity in mice such as behavioral changes, locomotion, convulsions, lost appetite and sneezing. The LD_{50} is greater than 5000 mg/kg.

DISCUSSION

The objective of this study was to determine the *in vitro* nematocidal activity of the ELE (ethanolic leaves extract) of *W. indica* against the bovine parasite, *O. ochengi* which is the closest relative to the human form, *O. volvulus* and the free living nematode *C. elegans* (wild-type and mutants). Thus, the results obtained revealed that both nematodes (*O. volvulus*, *C. elegans*) used in other earlier studies [17-20, 32, 42, 43] as models, are also sensitive to the ELE of *W. indica*. Mortality rates determined after 24 h, 48 h and 72 h incubation were concentration-dependent. These mortality rates reached 100% after 72 h for both nematodes, which justifies the lethal activity of the ELE of *W. indica*. There was no mortality recorded in 0.5% of DMSO used as negative control. Activity of ELE of *W. indica* had never been done on the bovine parasitie *O. ochengi* and *C. elegans*. However, other studies have justified its efficacy against bacteria, plasmodium, trypanosoma and viruses [44-47]. In another study, Maregesi *et al.,*[48] reported with the same plant an IC_{50} of water-methanol (80%) root extract ranging from 125 to 250 mg/ml against *Plasmodium falciparum*. Elsheikh *et al.,*[49], reported that the aqueous leaves extract was also lethal against miracidia of *Shistosoma mansoni*. In addition, Ragasa *et al.,*[50], demonstrated that the 5, 2′, 5′-trihydroxy-3, 7, 4′-trimethoxyflavone, a flavonoid isolated from *W. indica* is lethal to *Candida albicans* (sometimes responsible for diarrhea inhumans).

The remarkable activity of *W. indica* (ELE) against bacteria and viruses has been attributed to the action of tannins by some authors. Miyashiro *et al.,*[51], demonstrated that tannins were responsible of the lethal activity of *W. indica* against bacteria. In another study, Miyashiro *et al.,*[51], showed also that tannins may selectively inhibit HIV replication and are widely known to make trees and shrubs a difficult meal for caterpillars due to its astringent taste. Lethal activity of the ELE of *W. indica* against *O. ochengi* and *C. elegans* could be also due to the action of tannins or others bioactive compounds. It has been demonstrated that tannins interfere with the production of energy in helminth parasites by decoupling the phosphorylation oxidative [52]. Another possible anthelmintic effect of tannins is that they can bind to glycoproteins on the cuticle of the parasite and can cause death [53]. Other secondary metabolite able to act on nematodes could be flavonoids as reported in a study by Braguine *et al.,*[54]. Moreover, it has been demonstrated by Obasi *et al.,*[55] that flavonoids have the ability to affect pathogen microorganisms. According to Mohd *et al.,*[56], it is possible that helminths possess different susceptibility to cardiac glycosides which are presents in the ELE of *W. indica*. In addition, another study reported that cardiac glycosides are known to act by inhibiting the (Na^+/K^+) pump, thereby increasing the amount of Ca^{2+} ions available for the contraction [53].

The phytochemical analysis of the ELE of *W. indica* has revealed the presence of tannins, phenolic acids, saponins and flavonoids and the absence of alkaloids. These results are similar to those found by Zongo *et al.,*[52]. Contrary to Loustalot and Pagan [57], alkaloids were found in leaves of *W. indica*. Saponins (12.39 ± 1.00 mg/g) were the most quantified compound followed by tannins (0.96 ± 0.07 mg GAE/ 100 g), phenolic acid (0.51 ± 0.07 mg/g GAE), and flavonoids (0.0 4 ± 0.07 mg of rutin/100 g).

Ivermectin and levamisol used as positive controls were lethal against both nematodes. The difference was significant between LC_{50} values of ivermectin, levamisole and those of the ELE of *W. indica* on Wild-type of *C. elegans* after 72 h of incubation. These results indicate that plant extract could also act on nematodes like drugs. Despite the different resistance conferred to free living strain of *C. elegans*, the ELE of *W. indica* was potentially effective. The synergic activities of secondary metabolites present in plant extract could be responsible of his effect on resistant strains. Ivermectin and levamisole are recognized drugs and their mode of action is known. According to Yates *et al.,*[58], ivermectin acts on glutamate gated chloride channels (GluCls) which are members of the ligand-gated ion channel superfamily that includes nicotinic acetylcholine receptors (nAChRs). It has been reported by Hrckova and Velebny [64], that levamisole kills the

parasite by a hyper contraction of muscles due to prolonged activation of the excitatory nicotinic acetylcholine (nACh) receptors on body wall muscle.

It has been reported by Rajesh *et al.,*[59], that the activity of a plant extract depends on the availability of bioactive compounds. These (secondary metabolites) are known to possess activity against pathogenic organisms. Hrckova and Velebny [60], mentioned also that in the nervous system of nematode, the plant extract is able to cause the hyperpolarization of cells by increasing the permeability to chloride ions through the cell membrane, and as a result, the worms are paralyzed and die.

Acute toxicity of the ELE of *W. indica* was evaluated on mice. Toxic clinical manifestations were the behavioral changes, locomotion, convulsions, lost appetite and sneezing. The LD_{50} is greater than 5000 mg/kg. According to Teke and Kuete [61], the ELE of *W. indica* present practically lower toxicity. Hamidu *et al.,*[62], reported also that a fraction from an ethylacetate leaves extract of *W. indica* present lower toxicity (LD50 of 875 mg / kg) in mice when administered orally.

Conclusion

It appears from our results that ethanolic leaves extract of *W. indica* is lethal against *O. ochengi* and *C. elegans* (WT, CB211 lev-1(e211, VC722 glc-2(ok1047). The ethanolic leaves extract of *W. indica* is more lethal against *O. ochengi* than *C. elegans*. The remarkable lethal activity of our plant extract on both nematodes is justified by the presence of secondary metabolites quantified (tannins, flavonoids, saponins and phenolic acids). Our results confirm the use of *W. indica* by traditional healers for the treatment of human and livestock parasites. The LD_{50} of the ELE of *W. indica* is greater than 5000 mg/kg. Further studies have to be carried out to isolate, characterize and elucidate the structures of the bioactive compounds from *W. indica* for drug formulation.

Abbreviations

DMSO: dimethyl sulfoxide; O: *Onchocerca*, C: *Caenorhabditis*, PBS: phosphate buffered saline; SD: standard deviation, SPSS: Statistical Package for the Social Sciences, MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, NGM: nematode growth medium, OCP: Onchocerciasis Control Program, OECD: Organization for Economic Cooperation and Development, HNC: National Herbarium of Cameroon, RPMI: Roosevelt Memorial Park

Conflicts of Interest: The authors declare that they have no competing interests.

Ethical Approval: No applicable.

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