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Phytochemical composition and anthelmintic activities of *Ficus sycomorus* L. (Moraceae) on the bovine parasite *Onchocerca ochengi* and drug resistant strains of the free-living nematode *Caenorhabditis elegans*

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Abstract

The anthelmintic activity of ethanolic, methanolic and methanolic/methylene chloride extracts of leaves stem bark and root bark of *Ficus sycomorus* was assessed *in vitro* by using the cattle parasite nematode *Onchocerca ochengi* and *Caenorhabditis elegans*. Extracts were diluted to different concentrations in 0,5 % DMSO in the presence of parasites and incubated at 37 ° C. The anthelmintic activity was observed in terms of number of live worms after 72 h of incubation. The methanolic/methylene chloride and methanolic extracts of leaves and roots bark showed higher activity on *O. ochengi* with LC₅₀ values 0,45 µg/ml, 0,65 µg/ml and 0,22 µg/ml; 0,62 µg/ml, 0,55 µg/ml, 0,40 µg/ml respectively. Lower activity was observed with ethanolic extract of stem bark. LC₅₀ values were 17,5 µg/ml, 12,5 µg/ml and 30 µg/ml respectively for leaves, roots bark and stem barks after 72 h of incubation. The wild strain WT of *Caenorhabditis elegans* was sensitive with the methanolic extracts of the leaves of *Ficus sycomorus* and CB3474 (ben-1 (e1880) was not sensitive in the presence of the methanolic extracts of the stem bark of *Ficus sycomorus* with the LC₅₀ values 239.16 µg/ml and 872.13 µg/ml respectively. Tests with ivermectin have shown high efficiency on *Onchocerca ochengi* with LC₅₀ value of 1 µg/ml. These results confirmed the efficiency and the use of *Ficus sycomorus* against nematode infections by traditional healers, herdsman and pastoralists. According to our data, extracts of plant could be used as an alternative to fight Human and Bovine onchocerciasis.

Keywords: Anthelmintic activities, *Ficus sycomorus*, phytochemical composition, *Onchocerca ochengi*, *Caenorhabditis elegans*

1. Introduction

Onchocerciasis, also known as river blindness is a neglected tropical disease (NTDs). It is transmitted to humans by the bite of infected black flies of the genus *Simulium* [1-2]. The female worms produce up to 1500 microfilariae daily, which aggregate in the human lymphatic system, in subcutaneous and deep connective tissues. Onchocerciasis is the second leading cause of infectious blindness in the world and causes disfiguring skin disorders, severe itching, eye lesions and immune responses frequently lead to complete blindness [3]. In hyperendemic communities, the disease is also a serious obstacle to socio-economic development in sub-Saharan Africa [4].

Out of, 37 million people who are infected worldwide, 99 % live in Africa, 270,000 are blind, 500,000 are patients with severe visual impairment and 6.5 million people suffer from severe itching of the skin [5-6]. A rapid epidemiological mapping of onchocerciasis (REMO) survey undertaken in Cameroon revealed that about 50% of the rural population was at risk [7-8]. In hyperendemic communities, the blindness rate can reach 15% and up to 40% of adults may have severe eye nodules [9].

According to the Centre Pasteur of Cameroon, onchocerciasis is present in the ten regions of Cameroon with about nine million people at risk of infection, five million people are infected and 30,000 are blind (www.pasteur-yaounde.org).

To control human onchocerciasis several means have been used over the years. In 1974, the (OCP) which concentrated on eliminating the *Simulium* vector, used larvicides including organophosphorus and biological insecticides in controlled areas. Then the vector control used substances such as abate, chlorphoxime and pyraclopfos. These substances presented limits, namely the resistance of the blackflies, the pollution of the environment and these products were toxic for the biodiversity [10]. In 1995, the second principal mode was the African Program for Onchocerciasis Control (APOC) undertook the treatment of disease by mass drug

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administration (MDA) of ivermectin per semester, oral and single dose of 150 µg/kg [11].

Ivermectin (still called Mectizan) only has a microfilaricidal action. However, it has adverse side effects including fever, headache, encephalopathies, eye disorders and conjunctival haemorrhages [12]. Ivermectin shows upsurges or resistance in some communities in Ghana and Sudan [13]. Ivermectin also poses a problem of co-endemicity in the presence of *Loa loa* hypermicrofilaraemia. The duration of treatment is very long, and lasts about 15 years [14]. However, broad resistance to drugs and insecticides compromises the effectiveness of disease control. To circumscribe the phenomenon of resistance, it would be interesting to look for new alternatives based on medicinal plants. Phytomedicine derived from plants has shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infection [15]. In Africa, more than 80% of people relied on traditional medicine for their primary health care and more than 30% of the plant species have been used for this purpose [16-17]. *Ficus sycomorus* is a plant of drier areas, spreading savannah tree, up to 21 (max. 46) m [18]. It has a wide application in native medicine preparation such as diarrhea, scrofula, stomach disorders, lactation disorders, chest disorders, dysentery, cough, helminthiasis, infertility and sterility [19]. Several studies have indicated the presence of alkaloids, flavonoids, tannins, saponins and anthraquinones. Other studies have shown the antifalcemic property of the bark and leaf extracts of this plant, which contains phenolic compounds for the most part [20-21]. It had been reported that,

F. sycomorus exhibited antihemolytic and antilipidperoxidation activities [22], hepatocarcinogenesis and hepatoprotective activities [23], insecticides and acaricides activities [24], antidiabetic activity [25], antioxidative and antibacterial activities [26], anti-diarrheal activity [27], analgesic and anti-inflammatory activities [28]. *Onchocerca ochengi* is a filaria genetically closely related to the human parasite *Onchocerca volvulus* [29]. *O. ochengi* is transmitted by the same blackfly vector (*Simulium damnosum*) as *O. volvulus*. The present study was conducted to investigate macrofilaricidal properties of *Ficus sycomorus* *in vitro* against the bovine filarial worm.

2. Materials and methods

2.1 Study area

The work was done in the city of Ngaoundere, capital of the Adamawa region. Ngaoundere lies between the 7th degree 19 of Latitude North and the 13th degree 35 of Longitude East.

2.2 Plant material and chemical

Leaves, stem barks and roots bark of *Ficus sycomorus* were harvested in Wakwa (7°19'39" North, 13° 35' 04" East) of Ngaoundere the Adamawa region in July 2016 (Figure 1). The plant was identified by a botanist, Pr. Tchobsala of the Department of Biological Sciences, University of Ngaoundere, Cameroon. A voucher specimen N°8617 HNC was deposited in the National Herbarium of Yaounde-Cameroon. All chemicals were purchased from Sigma-Aldrich (Deisenhofen, Germany) (www.Sigma-Aldrich.com).

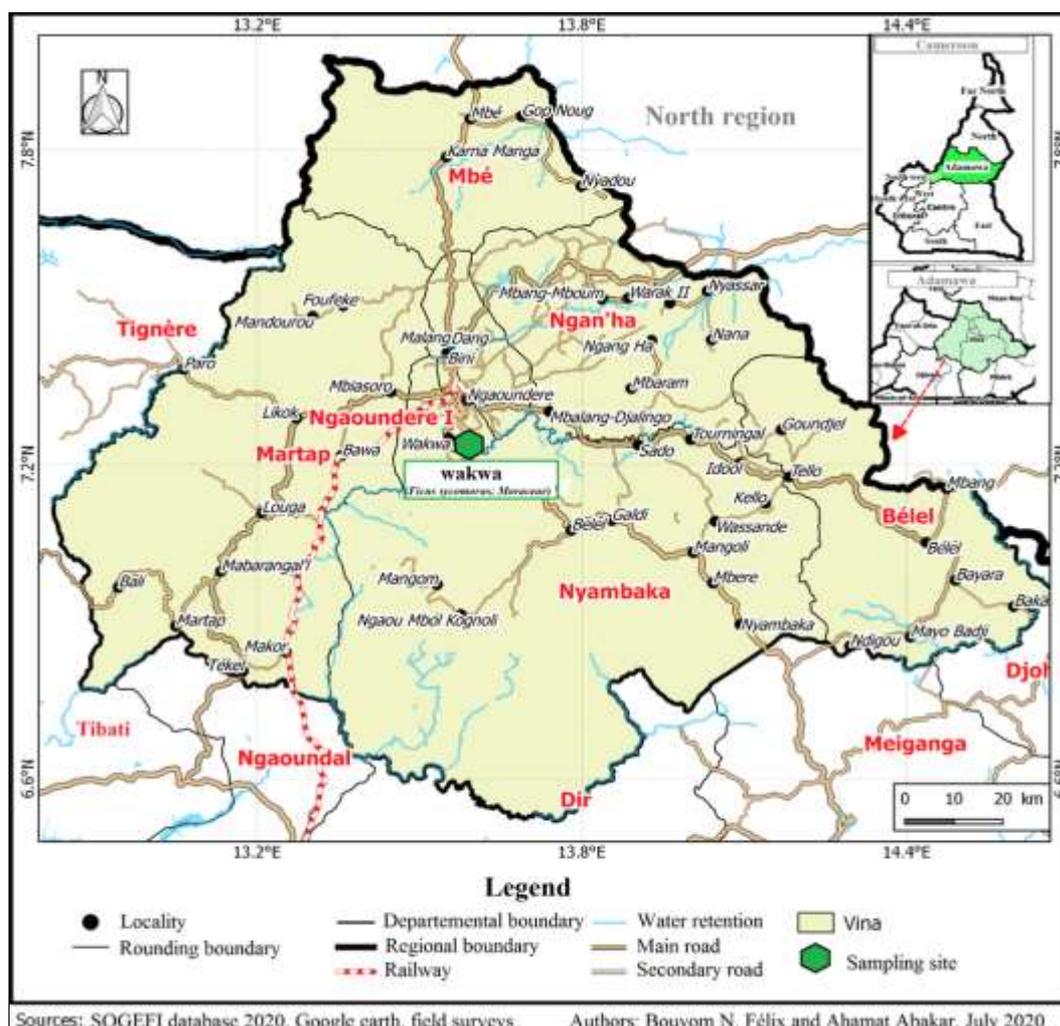


Fig 1: Map of Ngaoundere-Cameroon showing study area.

2.3 Preparation of plant extracts

Fifty grams of powdered plant material of *F. sycomorus* was macerated in 500 ml of EthOH-distilled water (70:30), MeOH-distilled water (70:30) and MeOH-CH₂Cl₂ (50:50 v/v) for 48 h at room temperature and centrifuged at 3500 rpm for 10 min. The supernatant was recovered and filtered using Whatman#1 filter paper then placed in an oven at a temperature between 40-50 °C to evaporate. The residue was weighed, transferred in a container and kept airtight for storage at 4 °C until further use [30-31].

2.4 Isolation and *In vitro* assay of *Onchocerca ochengi*

The isolation of *O. ochengi* adult worms was done following the method used by Ndjonka *et al.* [31]. Fresh pieces of umbilical cattle skin with palpable nodules bought from local slaughterhouse were brought to the laboratory for dissection, washed, drained and sterilized with 70% ethanol. Nodules of *O. ochengi* were removed from the skin of cattle. *O. ochengi* adult worms were carefully scraped out of the nodules as single masses, isolated and washed three times in sterile phosphate-buffered saline (PBS).

2.5 Monoxenic culture, synchronization and assays of *Caenorhabditis elegans*

The different strains of *C. elegans* used are: *C. elegans* wild type-N2 Bristol, the ivermectin-resistant strains VC722 (*glc-2(ok1047)* I) and DA1316 (*avr-14(ad1302)* I *avr-15(ad1051)* *glc-1(pk54)*), levamisole-resistant strains CB211 (*lev-1(e211)* IV) and the albendazole-resistant strain CB3474 (*ben-1(e1880)* III) were obtained from the *Caenorhabditis* Genetic Centre (CGC, Minneapolis, MN, USA). Strains were maintained at 20 °C on nematode growth medium (NGM: 2.5 g peptone from casein, 3 g NaCl, 17 g agar, 0.5% cholesterol, 1 mM CaCl₂, 1 mM MgSO₄, 25 mM KH₂PO₄/K₂HPO₄ in 1 l of distilled water) in Petri dishes seeded with *Escherichia coli* OP50 to serve as food source. Petri dishes of 85 mm with gravid adult worms were selected for age-synchronization [14-31]. *C. elegans* worms age-synchronized were obtained from eggs and isolated by bleaching [32]. The content of the plate was rinsed for 5 minutes. The supernatant was recovered in eppendorf tubes then centrifuged at 8000 rpm for 1 min at 4°C. The supernatant was discarded and completed with chlorox and mixture for 8 min. Then, the mixture was centrifuged at 8000 rpm for 1 min at 4°C. The supernatant was discarded and the pellet with viable eggs was rinsed 3 times in 1ml M9 buffer. During each rinse, the mixture was centrifuged at 8000 rpm for 1 min 4°C. The pellet was kept in 200 µl M9 buffer during the last rinse and then introduced in axenic liquid medium (3% (w/v) yeast extract, 3% (w/v) soy peptone, 1 % (w/v) glucose, 0.5 mg/ml cholesterol and 0.5 mg/ml bovine hemoglobin) supplemented with 100 U/mL penicillin and 100 g/ml streptomycin. After 48 hours of incubation at 20°C, the young adults or L4 larvae of the same age were used in 24well plates to test plant's toxicity [31-33]. The synchronized worms (ten young L4 adults per well) were transferred and incubated in M9 buffer with different concentrations of plant extracts or drug in the 24-well plates. Each well received 500 µl of M9 buffer to which the volume of extract's stock solution corresponding to each concentration was added. Negative control plates did not contain plant extracts or drug. Positive controls were performed with ivermectin, albendazole and levamisole. 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 [34-35].

2.6 MTT-formazan colorimetric assay for viability of worms

The MTT (3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide) colorimetric test is an *in vitro* test determining the viability of worms. This viability assessment test is based on the ability of living cells to reduce MTT, yellow in its metabolite formazan blue (purple color). The worms were placed in 24-well plate containing 500 µl of 0.5 mg/ml MTT in PBS in incomplete RPMI culture medium, and then incubated in dark at 37°C under the incubator à CO₂ (5%) for 30 minutes under a binocular microscope. Thus, alive worms were colored in 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.

2.7 Phytochemical analysis

The powdered form of *Ficus sycomorus* leaves, stem bark and roots bark were used to screen active compounds by using standard methods [36-37].

2.8 Experimental animals

Mice (*Mus musculus*) weighing between 25 and 30 g were used for acute toxicity test. Male and female albino rats (*Rattus norvegicus*) between 8 and 12 weeks, weighing 130–160 g were used for subacute toxicity study. The animals were obtained from LANAVET (National Veterinary Laboratory, Garoua-Cameroon). These animals were kept in polypropylene cages under identical animal house condition and provided with standard pellet and water ad libitum. The animals were maintained at a temperature of 22°C ± 2°C, with a 12 h light/dark cycle and a relative humidity of 60% ± 10%. All animal related experimental procedures were approved by the regional delegation of Livestock, Fisheries and Animal industries (N°20/17/L/RA/DREPIA).

2.9 Acute toxicity

The acute oral toxicity test was conducted in compliance with OECD guideline 423 which stipulate the use of only three animals per dose (OECD 423, Paragraph 23) [38]. Mice of both sexes were used. The mice were acclimatized to the standard laboratory conditions one week before starting the experiment.

Three groups of three animals were kept fasting overnight (12 h) and weighed. Test doses were calculated in relation to the body weight. Every fasting animal was administered drug via oral gavage.

2.10 Subacute toxicity

Sub-acute toxicity test was carried out according to OECD 407 guidelines [39]. Rats were divided into 4 groups of 10 each (5 males and 5 females). The control group was treated with distilled water and the other groups were administered the plant extract at the increasing doses of 250, 500 and 1000 mg/ml body weight. The extract was administered by oral route daily for 4 weeks. During this period, the animals were observed for clinical signs and symptoms, behavior alteration, food and water intake and body weight changes recorded at the end of each week. The last day of treatment, the animals were placed individually in metabolic cages for 24 h. The survivors were anesthetized by an intraperitoneal injection of ketamine with at 50mg/kg then sacrificed. The blood samples were collected into heparinized and non-heparinized tubes centrifuged at 3000 rpm for 15 min for hematological analysis and biochemical analysis. The serum obtained as supernatant, was collected in an eppendorf tube and kept at 4°C till use.

The vital organs liver, kidney, lung, heart, spleen, testis, epididymis, uterus and ovary were removed, cleared of the fat material, rinsed with saline at 0,9 %, weight, observed for any gross lesions and stored at -20 °C for biochemical analyzes. The organs preserved in 10% neutral buffered formalin for histological analysis. The organ index is a parameter that allows knowing the influence of the food taken and treatment on the condition of the organs after the experiment [40]. The relative organ weight of each animal was then calculated according to the formula:

$$Pr = \frac{Po}{Pa} \times 100$$

Pr: relative organ weight (g/100 g);
 Po: absolute organ weight (g);
 Pa: body weight of rat on sacrifice day (g).

2.11 Hematological analysis

After collecting blood from cardiac puncture into EDTA containing tubes, various parameters were evaluated at the regional hospital of Ngaoundere-Cameroon. Determination of white blood cells, red blood cells, hematocrit, platelets, hemoglobin, neutrophils, basophils, eosinophils, lymphocytes, monocytes, mean corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were analyzed using automaton hematology (Mindray, BC-2800).

2.12 Biochemical parameters analysis

Biochemical parameters including creatinin, glucose, total

protein (TP), alanine transaminase (ALT), aspartate aminotransferase (AST), and uric acid were analyzed in serum by different assay kits following the manufacturer protocol. These analyses were performed using spectrophotometer (Mindray, BA-88A).

2.13 Statistical analyses

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

3. Results

3.1 The effect of *Ficus sycomorus* methanolic/methylene chloride extracts on *Onchocerca ochengi*

The plant extracts inhibited the mortality of *O. ochengi* adult and *C. elegans* a time and concentration-dependent. The anthelmintic activities of leaves, trunk bark and root bark of *F. sycomorus* on *O. ochengi* adult and on *C. elegans* WT were evaluated in terms of mortality after 24h, 48 h and 72 h of incubation.

The curve illustrates the effect of the methanolic extract of the leaves of *F. sycomorus* on the adult males of *O. ochengi*, since in the presence of this extract the mortality rate of these worms increases with increasing time and concentration. Thus, we find that after 48 hours of incubation at 0, 25 µg / mL mortality reached 100% (Figure 2). So, we can say that the methanolic extract has a potentiating effect at low concentration on *O. ochengi*.

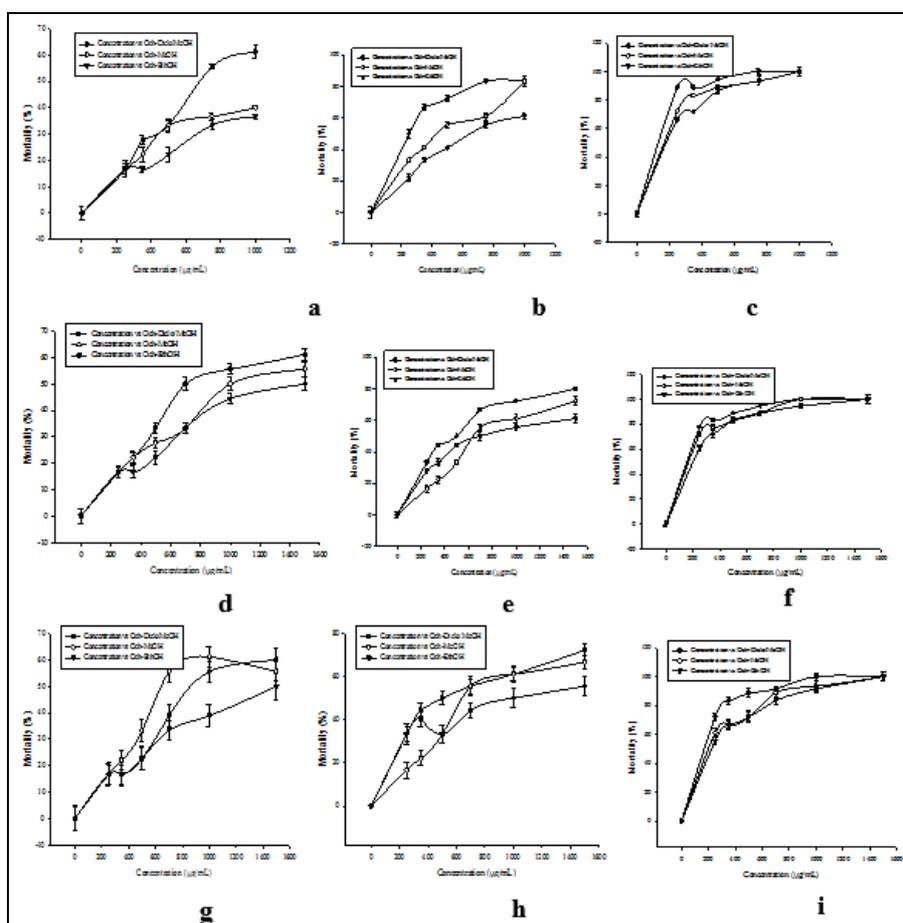


Fig 2: The methanolic/methylene chloride, methanolic and ethanolic extract of *Ficus sycomorus* on *Onchocerca ochengi* after 24 h (a: Root bark, d: Leaves, g: Stem bark), 48 h (b: Root bark, e: Leaves, h: Stem bark) and 72 h (c: Root bark, f: Leaves, i: Stem bark) of incubation. Data are mean ± SD from three independent duplicate experiments.

3.2 Inhibition assessment of *Ficus sycomorus* on *Caenorhabditis elegans* wild type and mutants

The present experiment consisted of evaluating the anthelmintic activity of the ethanolic, methanolic and methanol / methylene chloride extract in relation of the positive controls on the wild strain (WT) of *C. elegans*. The various curves in figures 3, 4 and 5 showed the influence of these crude extracts used on the adult mortality rates of the wild strain as a function of time and concentrations. In addition, the anthelmintic activity of these same extracts

was evaluated on mutants resistant to ivermectin (DA1316; VC722), albendazole (CB3474) and levamisole (CB211) of *C. elegans*. Figure 2, 3 and 4 shows the mortality curves of the mutants incubated in the presence of the crude extracts (Figure 2, 3 and 4). At 48 h of incubation, the mutants showed concentration-dependent sensitivity. Thus the values affecting half of CB4374, DA1316, VC722 and CB211 were $435.38 \pm 18.19 \mu\text{g/mL}$, $151.47 \pm 2.12\mu\text{g/mL}$, $438.53 \pm 13.60\mu\text{g/mL}$ and $226.91 \pm 4.25\mu\text{g/mL}$ respectively.

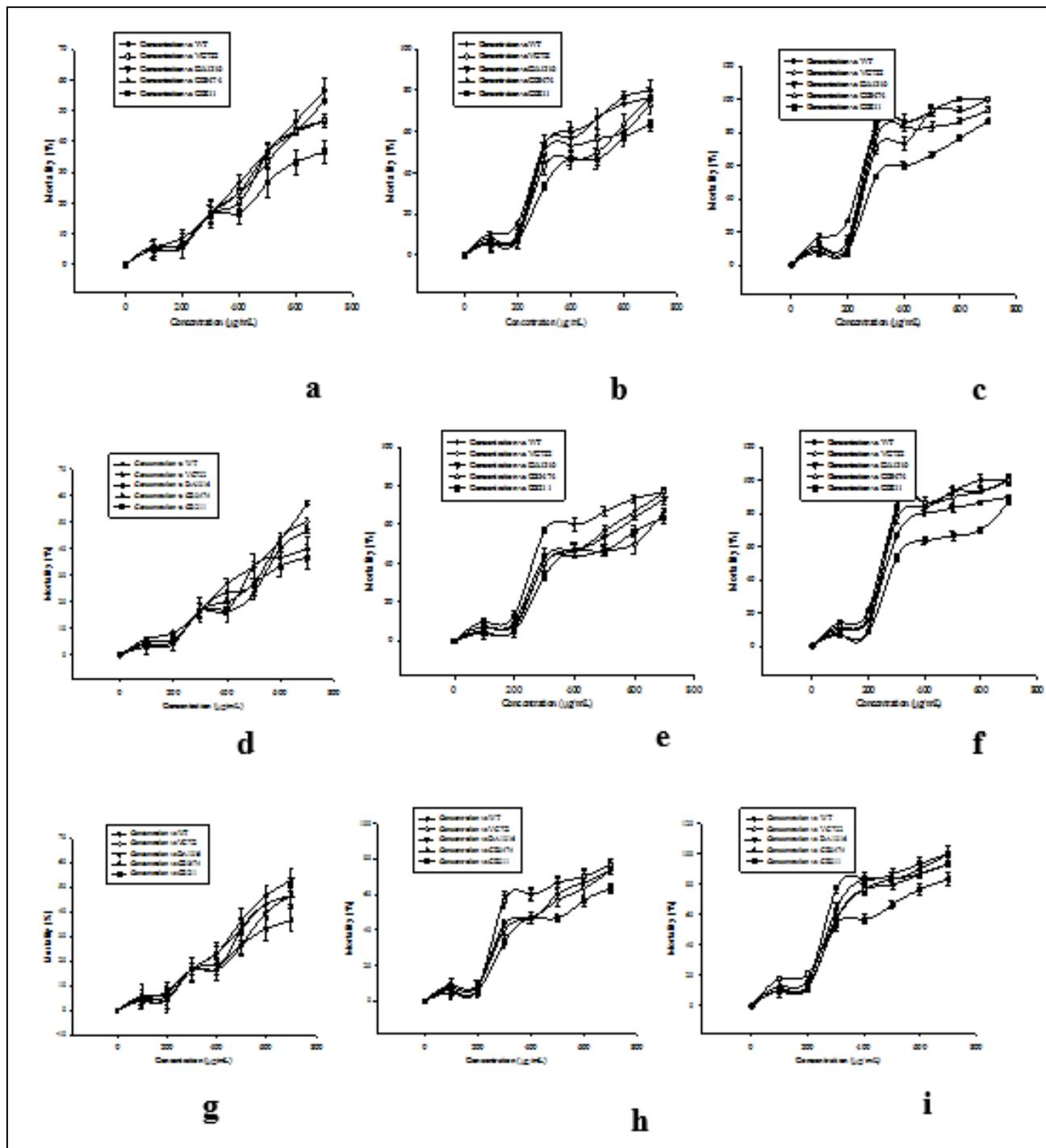


Fig 3: The methanolic/methylene chloride extract of *Ficus sycomorus* wild type WT (●) of *C. elegans* and CB211 (■) strain resistant to levamisole, CB3474 (Δ) resistant to albendazole, DA1316 (▼) and VC722 (○) resistant to ivermectin after 24 h (a: Root bark, d: Leaves, g: Stem bark), 48 h (b: Root bark, e: Leaves, h: Stem bark) and 72 h (c: Root bark, f: Leaves, i: Stem bark) of incubation. Data are mean ± SD from three independent duplicate experiments.

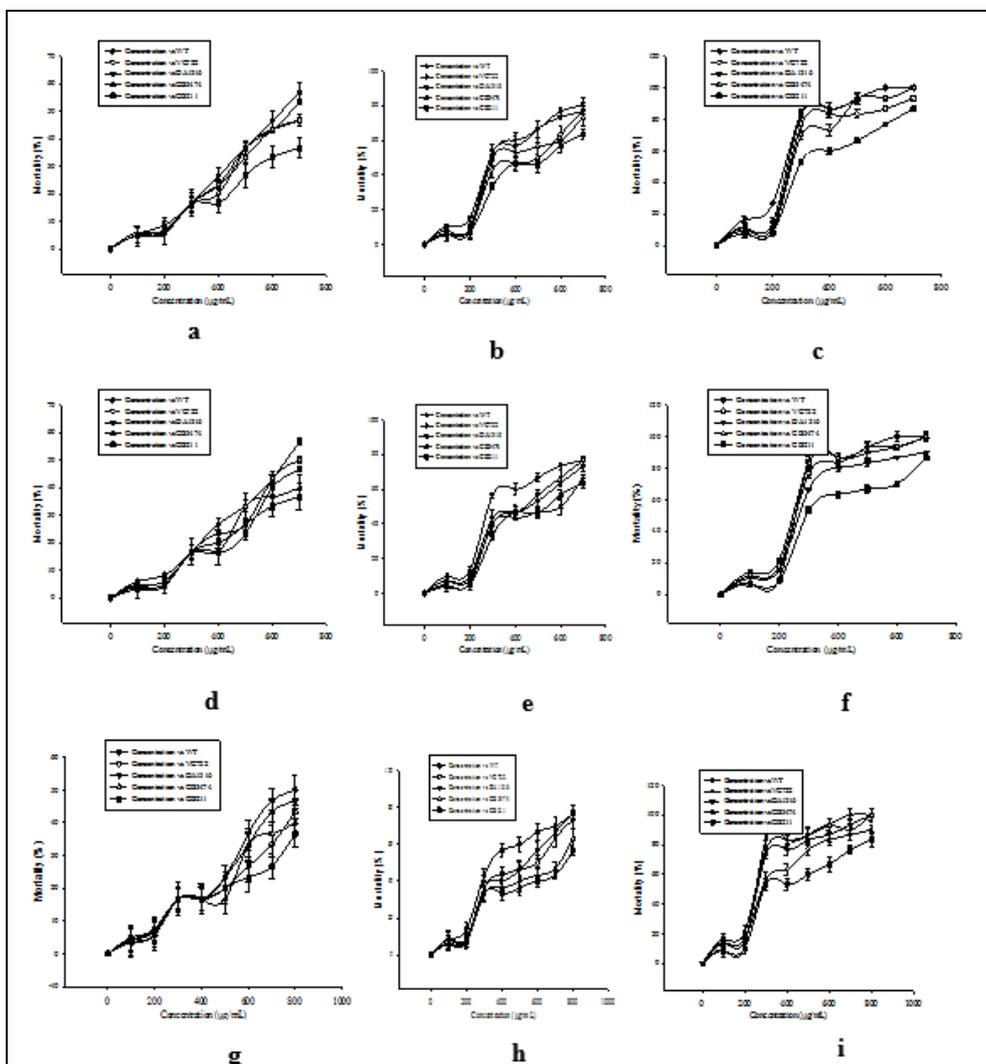


Fig 4: The methanolic extract of *Ficus sycomorus* on wild type WT (●) of *C. elegans* and CB211(■) strain resistant to levamisole, CB3474(Δ) resistant to albendazole, DA1316 (▼) and VC722 (○) resistant to ivermectin after 24 h (a: Root bark, d: Leaves, g: Stem bark), 48 h (b: Root bark, e: Leaves, h: Stem bark) and 72 h (c: Root bark, f: Leaves, i: Stem bark) of incubation. Data are mean ± SD from three independent duplicate experiments.

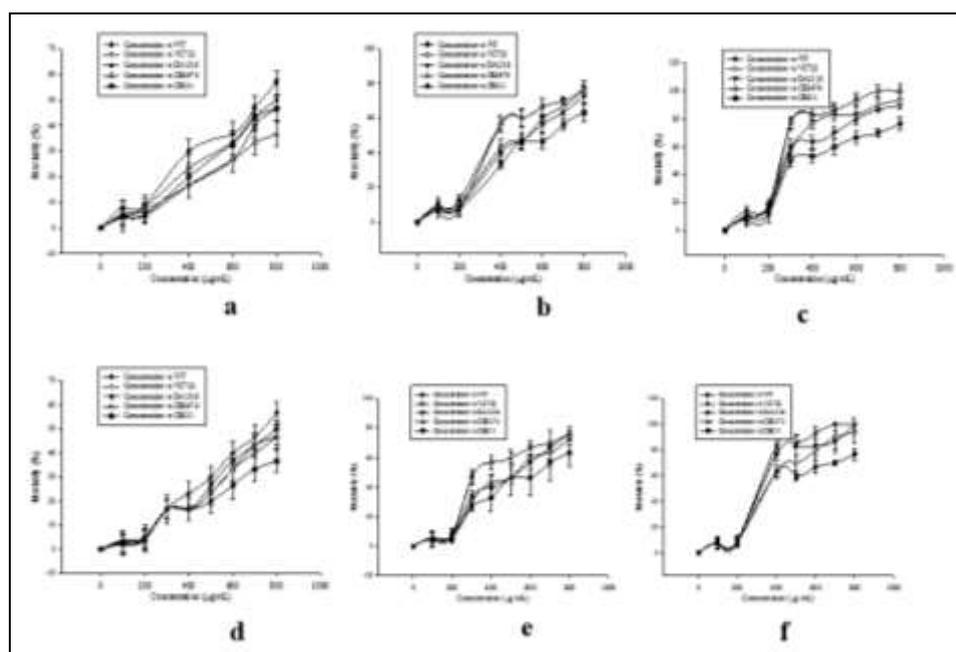


Fig 5: The ethanolic extract of *Ficus sycomorus* on wild type WT (●) of *C. elegans* and CB211(■) strain resistant to levamisole, CB3474(Δ) resistant to albendazole, DA1316 (▼) and VC722 (○) resistant to ivermectin after 24 h (a: Root bark, d: Leaves, g: Stem bark), 48 h (b: Root bark, e: Leaves, h: Stem bark) and 72 h (c: Root bark, f: Leaves, i: Stem bark) of incubation. Data are mean ± SD from three independent duplicate experiments.

Thus, the methanolic and methanol / methylene chloride and ethanolic extract of leaves of *F. sycomorus* and ivermectin

showed 100% mortality of the wild strain (WT) of *C. elegans* after 48 h of incubation at 37 °C.

Table 1: Quantity (mg) of phytochemical compounds (tannins, phenolics, flavonoids and saponins)

Extracts	Ethanolic extract				Methanolic extract				Methanolic / Methylene chloride extract			
	Tannins (mg/GAE)	Phenolics (mg/GAE)	Flavonoids (mg/GAE)	Saponins (mg/GAE)	Tannins (mg/GAE)	Phenolics (mg/GAE)	Flavonoids (mg/GAE)	Saponins (mg/GAE)	Tannins (mg/GAE)	Phenolics (mg/GAE)	Flavonoids (mg/GAE)	Saponins (mg/GAE)
Leaves	271.88 ± 5.28	97.89 ± 8.60	124.23 ± 6.92	89.36 ± 9.38	300.62 ± 4.46	93.61 ± 7.41	46.90 ± 8.36	189.93 ± 12.76	214.74 ± 4.74	790.44 ± 9.40	122.11 ± 4.74	70.68 ± 5.28
Rootbark	194.05 ± 3.97	343.94 ± 8.80	107.85 ± 7.78	226.98 ± 7.15	227.06 ± 5.28	113.17 ± 5.82	7.17 ± 3.60	227.91 ± 21.16	199.14 ± 6.10	649.75 ± 9.48	90.22 ± 8.35	321.33 ± 11.81
Stem bark	175.66 ± 5.12	833.35 ± 9.10	100.73 ± 8.09	162.79 ± 5.28	172.05 ± 6.10	81.94 ± 6.56	47.42 ± 8.24	123.00 ± 9.02	115.89 ± 5.61	725.80 ± 9.38	63.59 ± 2.22	220.44 ± 13.69

Notes: For each 100 grams of the ethanolic, methanolic and methanolic/methylene chloride extracts of *Ficus sycomorus*. GAE: Gallic Acid Equivalent. Values are expressed as mean ± SD.

Table 2: LC₅₀ of *F. sycomorus* crude extracts and positive control tested against *O. ochengi* and *C. elegans* after 48 h exposure. Values are mean±SD from three independent duplicate experiments.

Worms	LC ₅₀ / 48 h (µg/mL)									Albendazole	Ivermectin	Levamisole
	Ethanolic extract			Methanolic/methylene chloride extract			Methanolic extract					
Parts of plant	Leaves	Stem bark	Roots bark	Leaves	Stem bark	Roots bark	Leaves	Stem bark	Roots bark			
Means LC ₅₀ ±SD												
<i>O. ochengi</i>	17,50 ± 1,50	30 ± 2,27	12,50 ± 0,50	0,62 ± 0,01 ^a	0,40 ± 0,00 ^a	0,55 ± 0,12 ^a	0,22 ± 0,01 ^a	0,65 ± 0,15 ^a	0,45 ± 0,03 ^a	2,28 ± 0,25	2,13 ± 0,19	2,09 ± 0,45
<i>C. elegans</i> WT	683,41 ± 61,62 ^b	657,07 ± 11,52 ^b	722,59 ± 11,20 ^b	629,03 ± 10,06 ^b	425,56 ± 7,84 ^b	320,33 ± 12,67 ^b	239,16 ± 14,05 ^b	551,94 ± 11,33 ^b	428,53 ± 8,26 ^b	/	/	/
<i>C. elegans</i> VC722	446,14 ± 18,64 ^a	711,37 ± 6,48 ^b	428,57 ± 2,99 ^b	278,22 ± 21,73 ^b	302,16 ± 23,12 ^b	256,18 ± 20,45 ^b	438,53 ± 13,60 ^b	531,89 ± 10,40 ^b	347,05 ± 5,04 ^b	Nd	>100	Nd
<i>C. elegans</i> DA1316	611,20 ± 8,21 ^b	573,56 ± 13,94 ^b	723,36 ± 145,44 ^{ab}	1623,05 ± 3,76 ^b	204,11 ± 5,52 ^b	173,90 ± 7,91 ^b	151,47 ± 2,12 ^b	436,13 ± 3,72 ^b	252,65 ± 6,66 ^b	Nd	>100	Nd
<i>C. elegans</i> CB3474	851,08 ± 2,91 ^b	420,29 ± 10,28 ^b	860,61 ± 15,26 ^b	847,09 ± 14,34 ^b	896,04 ± 11,84 ^b	826,17 ± 13,53 ^b	435,38 ± 18,19 ^b	872,13 ± 16,29 ^b	443,20 ± 3,84 ^b	>100	Nd	Nd
<i>C. elegans</i> CB211	575,03 ± 5,72 ^b	549,22 ± 11,16 ^b	631,21 ± 189,41 ^b	419,06 ± 9,03 ^b	494,14 ± 11,23 ^b	454,97 ± 10,29 ^b	226,91 ± 4,25 ^b	511,93 ± 10,98 ^b	257,19 ± 3,76 ^b	Nd	Nd	>100

3.3 Phytochemical analysis

A phytochemical analysis of the *F. sycomorus* revealed that alkaloids, tannins, flavonoids, saponins, triterpenes, polyphenol, coumarins, anthraquinones and steroids were present (Table 1). These results revealed that some active compounds present in this plant extract might be responsible for the nematocidal activity.

3.4 Acute toxicity studies

During the 15-day period of toxicity study, oral administration of methanolic/methylene chloride extracts of leaves of *Ficus sycomorus* at 2000 mg/kg and 5000 mg/kg according to 423 did not produce any deaths and clinical signs of toxicity. As there were no mortality and clinical signs of toxicity in both the tested doses.

3.5 Subacute toxicity studies

There were no treatment related toxicity signs and mortality observed in both sexes of rats treated at 250mg/kg, 500mg/kg and 1000mg/kg orally during the 4 weeks of treatment. No significant differences in body weight were observed between the initial and final body weight of the rats treated with methanolic/methylene chloride extracts of leaves of *F. sycomorus* and control rats (Table 1). A similar absence of toxic effect was observed in the case of food and water consumption. There were no significant differences between control and methanolic/methylene chloride extracts of leaves of *F. sycomorus* treated groups in organ weight. Kluwe ^[41],

showed that the increase in organ weight had been observed to be a relative sensitive indicator of nephrotoxicity. Thus, *F. sycomorus* did not induce any toxic effect on the kidneys and the other organs going by this indicator. An increase of food and water consumption was observed in the animal treated with extract and control.

3.6 Hematological parameters

Hematological parameters such as white blood cells (lymphocytes, granulocytes, and monocytes) red blood cells, hemoglobin, hematocrit and blood platelets have been determined as representing targets sensitive to food substances.

Hematological tests showed no significant differences in total red blood cell count, total white blood cell count, platelet count, hemoglobin, hematocrit and differential leukocyte count in both control and treated groups during the experimental period. The results of hematological analysis were not represented.

3.7 Biochemical estimations

The result of biochemical parameters in the group treated and control rats were presented in table 3. Daily administration of Methanolic/methylene chloride extracts of leaves of *Ficus sycomorus* did not show any significant changes in biochemical parameters such as creatinin, uric acid (Table 4), glucose, TP, AST and ALT, when compared to control groups.

Table 3: Effect of methanolic/methylene chloride extracts leaves of *Ficus sycomorus* serum biochemical parameters

Serum Biochemical parameter	Sex	Normal range	Treatment group			
			Control	250 mg/kg	500 mg/kg	1000 mg/kg
Glucose (mg/dL)	Males (n=5)	70-119	99.17 ± 6.21	97.28 ± 6.15	95.83 ± 6.12	102.88 ± 5.78
	Females (n=5)		97.19 ± 6.11	98.38 ± 6.17	100.83 ± 6.07	103.58 ± 5.66
ALT (IU/L)	Males (n=5)	10-50	40.10 ± 2.32	44.00 ± 2.59	43.01 ± 2.25	42.60 ± 2.44
	Females (n=5)		39.20 ± 2.76	43.71 ± 2.53	45.07 ± 2.56	39.97 ± 2.51
AST (IU/L)	Males (n=5)	68-135	53.65 ± 3.16	54.51 ± 3.43	55.22 ± 3.28	54.44 ± 3.09
	Females (n=5)		51.45 ± 2.26	52.40 ± 3.86	49.11 ± 2.37	50.29 ± 2.98
TP (g/dL)	Males (n=5)	4.8-9.2	6.15 ± 0.12	6.17 ± 0.23	6.13 ± 0.24	6.11 ± 0.23
	Females (n=5)		6.18 ± 0.12	6.16 ± 0.14	6.13 ± 0.9	6.09 ± 0.15

Notes: Values are mean ± SEM, n=5 females and 5 males. Values for a given sex in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$).

Table 4: Effect of Methanolic/methylene chloride extracts leaves of *Ficus sycomorus* on biomarkers of kidney malfunction

Serum Biochemical parameter	Sex	Treatment group			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Creatinine (mg/dl)	Males (n=5)	0.84±0.17	0.85±0.12	0.87±0.13	0.84±0.18
	Females (n=5)	0.86±0.14	0.82±0.15	0.83±0.14	0.85±0.12
Uric acid (mg/dl)	Males (n=5)	4.34 ± 0.19	4.29 ± 0.20	4.21 ± 0.21	4.17 ± 0.19
	Females (n=5)	4.45 ± 0.21	4.37 ± 0.14	4.30 ± 0.18	4.23 ± 0.16

Notes: Values are mean ± SEM, n=5 females and 5 males. Values for a given sex in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$).

4. Discussion

The purpose of the present study was to investigate nematocidal molecules of natural product leads offer an efficient approach to discovering new drug for human disease use. Solvents of increasing polarity (ethanol, methanol/methylene chloride, and methanol) were used to produce three crude extracts from the leaves, stem bark and root bark of *Ficus sycomorus*. The methanolic extract of leaves of *F. sycomorus* was the most active on *O. ochengi* and *C. elegans* (wild type and drug resistant strains).

The nematocidal activity of *F. sycomorus* was assessed *in vitro* against four resistant strains of the free-living nematode *C. elegans*, namely CB211 resistant to levamisole, CB3474 resistant to albendazole, DA1316 and VC722 resistant to ivermectin. According to Hoste *et al.* [42], the anthelmintic activities of a plant extract depend on the availability of bioactive compounds, secondary metabolites such as tannins, saponins, terpenoids, alkaloids and flavonoids.

In our work, phenolic acids and tannins were the most abundant compound content with 790.44 ± 9.40 mg/kg and 300.62 ± 4.46 mg/kg respectively. Prashant *et al.* [43] reported that polyphenols and tannins have anthelmintic activities. The presence of these secondary metabolites might be responsible for the high nematocidal activity on the worms. Tannins present in their composition several phenolic groups such as ellagic, gallic and gentisic acids. Gallic and gentisic acids have been reported to be toxic for *C. elegans* and *O. ochengi* [44].

These results are similar to those of Mahmoudi *et al.* [45] who concluded that the solubility of phenolic compounds depends on their chemical nature in the plant, which varies from single to strongly polymerized compounds.

The effect of crude extracts of *F. sycomorus* on *O. ochengi* and *C. elegans* might be due to the presence of these phytochemical products which might act synergistically. The mortality observed in worms might be due to the presence of tannins in *Ficus sycomorus*, might be explained by the fact that tannins react directly with surface proteins of the worm.

Athanasidou *et al.* [46] reported that tannins can bind to free proteins or glycoproteins present on the cuticle of the parasite in the gastrointestinal tract of the host animal and cause their

death.

The extracts from the plant inhibit the mortality rate of *O. ochengi* adult male the wild strain of *C. elegans* WT and CB211 resistant to levamisole, CB3474 resistant to albendazole, DA1316 and VC722 resistant to ivermectin depending on the concentrations of extracts and the incubation time. Variations in the mortality rate reveal that the mortality rate of worms was reached 100 % after 48 hours of incubation with ethanolic and methanolic extract of *F. sycomorus*; however, this rate was reached 100 % faster with methanolic extracts of leaves of *F. sycomorus* (Figure 3).

Table 2 presents the LC₅₀ of extracts of *F. sycomorus* and reference drugs on *O. ochengi*, the wild strain of *C. elegans* and CB211 resistant to levamisole, CB3474 resistant to albendazole, DA1316 and VC722 resistant to ivermectin after 48 h of incubation.

The highest activity was recorded with the methanolic extracts of leaves of *F. sycomorus* on *O. ochengi* and with the values of LC₅₀ respectively (Table 2). Compared to wild type, mutant DA1316 is sensitive when incubated with methanolic extracts of leaves and methanolic / methylene chloride extracts of roots bark of *F. sycomorus* (Table 2).

In general, the ethanolic extract of *F. sycomorus* was the least effective on *C. elegans* with LC₅₀ values ranging from 5.05 µg / ml (± 1.57) to 370.10 µg / ml (± 0.08).

The ANOVA analysis of variance shows that there is a significant difference between the crude extracts of leaves, stem bark and root bark of *Ficus sycomorus* at the three fold of 5% ($P < 0.05$).

Ivermectin (22-23 dihydro-avermectin B1) acts on the glutamate-dependent chloride channels of invertebrates [47]. The 16-ring macrocyclic lactone acts by inhibiting the neurotransmission of glutamate or GABA residues, by affine binding to the glutamate-dependent chloride channels specifically present in nerve and muscle cells of invertebrates. This fixation leads to an increase in membrane permeability to chloride ions [44]. The cell is hyper polarized. This results in paralysis which causes the death of the parasite [48]. VC722 is a single mutant in which the GluCl subunit *glc-2* has been knocked out. Glc-2 represents the binding site of ivermectin in pharyngeal muscle cells [49]. The plant extract might 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. The triple mutant DA1316 (*glc-1*, *avr-14*, *avr-15*) is not similar to GluCl subunit *glc-1*, *avr-14* and *avr-15* together. This mutant is able to confer a 4000-fold reduction in sensitivity to ivermectin, whereas double mutant only shows either moderate or no resistance. These two mutants shown that the mechanism of action of the particle compounds of the *F. sycomorus* extract differs from that of ivermectin.

A levamisole-resistant strain developed from a wild-type strain was previously used by [50]. Levamisole acts by paralyzing the muscles of sensitive nematodes. Specifically, it increases the axial muscle tone of the worm, causing paralysis and its elimination [51]. CB211 is a knockout mutant of the gene *lev-1* which is expressed in body wall muscle and plays a role in egg-laying regulation and normal locomotion. Compared to WT, mutant CB211 is sensitive to the crude stem bark of methanolic/methylene chloride extract of LC₅₀ values $103,95 \pm 4,27 \mu\text{g/ml}$ at 48 h for incubation.

Albendazole is an anthelmintic drug used for several decades against gastrointestinal worms. Its fixation on β -tubulin leads to the inhibition of the formation of microtubules of the cytoskeleton [52-53]. These microtubules, which are involved in mitosis, nutrient absorption, secretion, intracellular transport and cell mobility, induce worm paralysis and a reduction in growth. The β -tubulin is encoded by the allele *ben-1* [54]. Since the mutant strain CB3474 *ben-1*(e1880) is slightly more resistant to the *F. sycomorus* extract than *C. elegans* wild type worms, we suggest that the plant extract might contain active compounds that act similarly to albendazole. Compared to wild type, mutants CB3474 is slight sensitive when incubated with root bark of *F. sycomorus*. This result suggests that the mode of action of root bark extracts of *F. sycomorus* may act similarly to albendazole. CB3474 is resistant when tested with leaves of *F. sycomorus*.

5. Conclusion

The present study assessed of methanolic/methylene chloride, methanolic and ethanolic extracts of *Ficus sycomorus* on bovine parasite *Onchocerca ochengi* and drug resistant strains of the free-living nematode *Caenorhabditis elegans*. The acute toxicity of crude extracts was tested on mice and the sub acute toxicity on rats. Hence may provide a source of a new antifilarial lead compound. Our results confirmed the efficiency and the use of *Ficus sycomorus* by traditional healers, herdsman and pastoralists in the treatment of the human onchocerciasis and other worm infections. Further studies have to be carried out to isolate, characterize and optimize the structures of the bioactive substances from *F. sycomorus* for pharmaceutical drug formulation.

6. Acknowledgements

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Consent

It is not applicable.

Ethical approval

This study was carried out in accordance with the Animal Ethical Committee No: 20/17/L/RA/ DREPIA of the Ngaoundere Regional Delegation of Livestock; Fisheries and Animal Industries, Cameroon.

Conflicts of interest

The authors declare that they have no Conflict of interest

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