

Anthelmintic Activity of Ethanolic Extract of Fruit Pulp of *Adansonia digitata* L. (Malvaceae) against *Onchocerca ochengi* and *Caenorhabditis elegans*

Ndouwé Tissébé Menga Honoré^{1*}, Kalmobe Justin², Dikti Vildina Jacqueline³, Ndjonka Dieudonné³

¹Department of Biological Sciences of Living Organisms, University of Garoua, P.O. Box: 346, Garoua, Cameroon

²Department of Parasitology and Parasitic Pathology, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon

³Department of Biomedical Sciences, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon

*Corresponding author: Ndouwé Tissébé Menga Honoré | Received: 30.01.2026 | Accepted: 26.03.2026 | Published: 01.04.2026 |

Abstract: In the present work, an ethanolic extract of *A. digitata* has been tested *in vitro* against the bovine parasite *Onchocerca ochengi* and *Caenorhabditis elegans*. The both nematods were incubated in different concentrations of the plant extract solubilized in RPMI 1640 supplemented with penicillin/streptomycin (100 U/mL/100µg/mL) and on NGM-agar. Ivermectin, Levamisole were used as the positive controls. The worm viability was determined by MTT/formazan colorimetry method after 24, 48 and 72 h. The oral acute toxicity on rats (60-120g) of the fruit pulp and its phytochemical composition were also assessed. The LC₅₀ values were determined using Probit Analysis and data were expressed as mean ± standard deviation. The extract was active on macrofilariae of *O. ochengi* and all *C. elegans* living strains. On *O. ochengi*, the LC₅₀ values for ethanolic FPE were 36.01, 12.03 and 5.05 µg/mL respectively after 24, 48 and 72 h. *C. elegans* strains were sensible to the FPE. The calculated LC₅₀ were 82.08, 60.02 and 49.01 µg/mL for the wild-type; 209.03, 151.21 and 95.61 µg/mL for CB211 strain; 370.10, 224.0.3 and 210.06 for VC722 strain respectively after 24, 48 and 72 h. Among the phytochemical constituents of the fruit pulp tannins (43.88 mg/kg GAE) were the most abundant followed by phenolic acid (21.28 mg/kg GAE), saponins (12.99 mg/kg) and flavonoids (0.07 mg of rutin/100g). The oral acute toxicity of FPE assessed on rats showed that the LD₅₀ was above 5000 mg/kg suggesting no toxicity. The relative organ weights of liver of rats sacrificed for all group varied from to 3.94 ± 0.23g to 12.92 ± 0.61g. The group treated with CCL₄ showed the highest values of relative organ weight with a maximum of 12.92 ± 0.61g for males and 12.78 ± 0.54g females. The difference is significant (p-value = 0.001) between the ALAT and ASAT values founded in liver of rats treated with FPE when compared to those treated with CCL₄ (carbon tetrachloride), ivermectin and levamisole. However, not significant difference (p-value = 0.001) was observed between these values of ALAT and ASAT in negative control group and those of rats treated with FPE. These results therefore suggest that the FPE extract at the different test doses (1750, 2000, 5000 mg / kg) was not toxic to the liver of rats. This study suggests possibility of new source of compounds against human onchocerciasis.

Keywords: *Onchocerca ochengi* *Caenorhabditis elegans*, *Adansonia digitata*.

INTRODUCTION

Human onchocerciasis (river blindness) is a filarial disease caused by *Onchocerca volvulus* transmitted through the bites of blackflies of the genus *Simulium* [1]. In the human host, adult worms (macrofilariae) live subcutaneously and induce the formation of onchocercomas. Females of *O. volvulus* produce thousands of microfilariae per day [2] that migrate subcutaneously throughout the human body and

elicit skin-irritations and eye-lesions the latter leading up to the destruction of the cornea or posterior eye [3]. Different surveys indicate that this disease is dramatically underestimated and is the world's second leading infectious cause of blindness with over 37 million people now thought to be infected [4]. Close to 99% of all patients live in tropical Africa and more than 400,000 infected in central and South America [5]. According to the Cameroonian public health ministry in 2004, about 28% of the population is affected by the

Quick Response Code



Journal homepage:
<https://www.easpublisher.com/>

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Citation: Ndouwé Tissébé Menga Honoré, Kalmobe Justin, Dikti Vildina Jacqueline, Ndjonka Dieudonné (2026). Anthelmintic Activity of Ethanolic Extract of Fruit Pulp of *Adansonia digitata* L. (Malvaceae) against *Onchocerca ochengi* and *Caenorhabditis elegans*. *Cross Current Int J Agri Vet Sci*, 8(2), 54-63.

disease, with more than 1 million suffering from skin pathology. Onchocerciasis causes socio-economic problems and life-long human suffering [6]. Two major strategies employed in the control of onchocerciasis are mass treatment of infected people with ivermectin and the elimination of the *Simulium* vectors [7, 8]. Despite onchocerciasis having been successfully controlled in parts of West Africa by the Onchocerciasis Control Program (OCP), infection with *O. volvulus* remains an obstacle to public health in many other endemic countries [9-11]. A global eradication of the parasite by means of ivermectin alone appears not feasible [12]. However, the treatment may be given for undetermined period of time in order to effectively cure [13]. In addition, ivermectin the only drug currently recommended for treatment of the disease effectively kills microfilariae of *O. volvulus*, but not the adult worms (macrofilariae) to which its reduces fecundity and apparently shorten live span [14], which normally is up to 18 years in humans [15]. Nowadays, the emergence of ivermectin resistance in parasitic nematodes of veterinary importance raises serious concerns that this may be generalized to *O. volvulus* [16]. Therefore, the search for new and highly efficacious microfilaricides and macrofilaricides is imperative. Due to the lack of a laboratory host for the human parasite *O. volvulus*, model organisms are needed. In this study, *O. ochengi* and *C. elegans* were used as models for several reasons. *O. volvulus* and *O. ochengi* are evolutionary closely related and are transmitted to human and bovine host by the same blackfly vector *S. damnosum* [17]. The potential of *Caenorhabditis elegans* as a model organism to address questions in neurobiology, developmental biology, genetics and molecular biology was recognized [18]. In particular, the mode of action of anthelmintic drugs such as ivermectin and levamisole was elucidated on the molecular level by employing this one millimeter non-parasitic bacterial feeder [19-21]. Medicinal plant preparations have been identified as alternative remedies for several diseases [22]. About 50% of drugs used in modern medicine are of plant origin [23] and about 80% of Africa's population relies on medicinal plants for their health needs [24]. The development of drug resistance in human pathogens against parasitosis necessitates a search for new anthelmintic substances. Higher plants produce diverse secondary metabolites with different biological activities [25]. These natural products may be a source of compounds with anthelmintic effects and therefore possible candidates for the development of new drug [26]. Throughout Africa *Adansonia digitata* is a wonderful tree for most of indigenous people; some even consider it bewitched [27]. Almost all parts of the tree are used in traditional medicine in Africa although this varies from one community to another. *A. digitata* products (e.g. fruits, seeds, leaves, bark) contribute to the livelihood of many populations in Africa as it is a source of food, fibre and medicine [28-30]. Several properties of *A. digitata* have been previously mentioned in several others studies. The plant has anti-inflammatory, antipyretic, antimicrobial and analgesic activities [31].

Powdered leaves have anti-asthmatic and have anti-tension and antihistamine properties [28]. Leaves are also used for other conditions such as diarrhea, dysentery, ophthalmia and otitis media. Bark is used as a substitute for quinine to curb high fever and act as prophylactic measure for malaria as well [28]. Aqueous stem bark extract of *A. digitata* has a high anti-malarial activity [32]. In other study reported by Van Staden *et al.*, [33], *A. digitata* has showed considerable antimicrobial activity against *Bacillus subtilis*, *S. aureus* and *C. albicans*. Thus based on informations of the regular use by traditional healers and people for the treatment of parasitosis in the North region of Cameroon, *A. digitata* has been chosen after a screening for this study. In an attempt to contribute to anti-onchocercal drug development, we evaluated the *in vitro* filaricidal activities of ethanolic fruit pulp extract of *A. digitata* on *O. ochengi* and on the free living strains of the nematode *C. elegans* and the acute toxicity was also studied in rats.

METHODS

Sample Collection and Preparation of Plant Extract

Fruits of *A. digitata* were collected from Garoua area (latitude 09°23'9.46" N, longitude 13°20'18" E) and identified at the National Herbarium in Yaounde/Cameroon, where it was registered under the voucher number 42417/HNC.

Preparation of the extract was done according to the method described by Cho-Ngwa *et al.*, [34]. Briefly, the fruit pulp of *A. digitata* was harvested, dried in the shade at room temperature, crushed to fine-sized particles and sieved on a 0.5 mm mesh screen. A ratio of ten grams of the powdered material extracted with 100 mL of ethanol at 70 % was used, macerated for 48 h, centrifuged (3500 rpm, 10 min) and filtered over filter papers No. 413 (VWR International, Darmstadt, Germany). The clear filtrate was concentrated by a rotary evaporator (Buchi R-200, Switzerland) at 40°C under reduced pressure, solidified at -20°C and lyophilized. The resulting powder was weighed and stored at + 4°C. The dried fruit pulp extract (FPE) was dissolved in dimethyl sulfoxide (DMSO) and RPMI (Roosvelt Memorial Park Institute) to a final concentration of 100 mg/mL, filtered and aliquoted. Its nematicidal activity was determined on *C. elegans* strains and *O. ochengi*.

Preparation of Drug Solutions

Ivermectin and levamisole were dissolved in 10 % dimethyl sulfoxide (DMSO), diluted with RPMI or distilled water respectively for *O. ochengi* and *C. elegans* assays. As positive controls, ivermectin and levamisole were used at 2 mg/mL for RPMI-stock solutions and 5, 100 mg/mL respectively for distilled water-stock solutions. 0.5 % DMSO was used as negative control.

Isolation of Adult Worms of *Onchocerca Ochengi* from Nodules

The isolation of macrofilariae was done according to the method used by Ndjonka *et al.*, [35]. Briefly, skin biopsies from umbilical area containing palpable nodules were collected from the slaughtered cattle of the local slaughterhouse. On a clean laboratory bench, they were washed in distilled water, disinfected with 10 % Povidone-iodine (Galentic Pharma, Mumbai/India) to avoid any source of contaminations and washed with sterile PBS (phosphate-buffered saline). From isolated nodules, *O. ochengi* adult worms were carefully scraped out as single masses and worms isolated. Worms in good condition was ascertained by visual examination under microscope (Euromex, Holland). The fully motile and not wounded ones were kept for subsequent *in vitro* culture.

In Vitro Assay on Onchocerca Ochengi

The *in vitro* assay on *Onchocerca ochengi* was done according to the method used by Ndjonka *et al.*, [36]. The selected worms were washed three times in sterile phosphate-buffered saline (PBS) and two times in RPMI-1640 (Sigma, Diesendorf/Germany) supplemented with antibiotics (penicillin 100 IU/mL/streptomycin 100 µg/mL, Sigma, Diesendorf/Germany). They were then incubated at 37°C under 5% CO₂, in 96-wells microtitre plates (one worm per well in 100 µL medium) in RPMI 1640 containing different concentrations of ethanolic FPE or drug. The mortality was determined after 24, 48 and 72 h. Positive control was performed with ivermectin and each test was run in triplicate.

Culture of Caenorhabditis Elegans

The evaluation of drugs nematotoxicity was performed on different strains of *C. elegans* according to the method described by Ndjonka *et al.*, [36]. The wild type N2 Bristol (WT) and the mutant strains CB211 lev-1(e211) IV (CB211), VC722 glc-2(ok1047) I (VC722). Their culture was made as follows: worms were cultured 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% cholesterolin, 1 mM CaCl₂, 1 mM MgSO₄, 25 mM KH₂PO₄/K₂HPO₄ in 1 l of water) in petri dishes seeded with *Escherichia coli* OP 50 as food source. The synchronization of the *C. elegans* culture was done according to the method used by Johnson *et al.*, [37]. Succinctly, the culture was submitted to 12.5 % hypochlorite treatment (chlorox), the collected eggs were transferred in M9 buffer until hatching. The juvenile stage (synchronized) were then introduced on NGM-agar plates and incubated at 20°C.

Assays on Caenorhabditis Elegans

The assays on *Caenorhabditis elegans* was performed as previously described by Thomas *et al.*, [38]. Briefly, the synchronized worms (ten young L4 adults per Petri dish) were transferred and incubated with different concentrations of the FPE or drug on a dried solid NGM-Agar seeded with *Escherichia coli* OP50 (80 µL). Each small petri dish (35 mm in diameter) receives

3 mL of hot NGM-Agar (about 40 ° C), to which the volume corresponding to each concentration is mixed in a falcon tube. Control plates did not contain plant extract or drug. Positive controls were performed with levamisole and ivermectin and each test was run in triplicate. Worms were incubated at 20 ° C and the mortality was determined after 24, 48 and 72.

Determination of Worm Mortality

The death was determined by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) colorimetric assay which is a cell metabolic activity-based assay. The MTT is a pale-yellow compound which is reduced to a dark blue product; the formazan by the living cells of the worms. Single intact worm was placed in each well of a 48-wells plate (Falcon, UK) containing 505 µL of a solution consisting of 5 µL MTT (0.5 mg/mL) and 500 µL of RPMI 1640, then incubated at 37°C and observed after 30 min under a microscope. Thus after incubation, when the worms were alive they were colored blue because of the MTT which was reduced to formazan. In the other hand, when their color remains yellow there are dead. Dead worms do not reduce MTT to formazan but simply take up the yellow color of MTT. All the MTT assays except the observation were done in the dark since the MTT reagent is sensitive to light as described by Loveland *et al.*, [39].

Phytochemical Screening and Extraction Yield

The tannins determination was conducted according to the modified method of Schanderl [40]. 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 minutes at room temperature (25 to 30°C). The absorbance was then read at 640 nm.

The phenolic acids content was evaluated using the Folin-Ciocalteu modified method of Folin and Denis [41], 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 (UV-biowave 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 flavonoids were determined according to the method described by Wolfe *et al.*, [42], modified. 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 transferred 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 determination was conducted according to the modified method described by Schanderl, [40]. To 0.1 g of the extract, 1 mL of distilled water was added and vigorously shaken for 30 min. The height of foam was measured by a ruler and quantified like following: Saponin (mg) = [(0.432) (height of foam in cm after 5 to 10s) + 0.008] / (weight of sample in gram). Extraction yield (mass of extract/mass of raw material) was determined.

Acute Toxicity Study

The oral acute toxicity of ethanolic FPE of *A. digitata* was determined using the method of Adedapo *et al.*, [43]. Twenty-four healthy adult male and female albino rats weighing 60-120 g and 10 weeks old were used. The assay was conducted according to the OECD [44], guideline. The animals were obtained from LANAVET (National Veterinary Laboratory, Garoua, Cameroon). Before assays, they were acclimatized (two weeks) to the conditions of breeding of LANAVET. Rats were separated into 4 groups of 6 animals (3 males and 3 females) and identified by color tail marks. They have been deprived of standard food for 12 h and received orally using a feeding needle different doses of the extract (1750, 2000, 5000 mg/kg) corresponding to groups A, B,C and a control group (D) which received distilled water. All the animals were then allowed free access to standard food and water and observed at 2, 4, 24 and 48 h for any behavioral changes and thereafter twice daily for a continuous period of 14 days.

Relative Organ Weight of Rats

After the fourteenth day, the rats were starved for 24 hours then they were euthanized under chloroform (10%) anesthesia. The liver were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated according to the following formula:

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Level of ASAT and ALAT Determination in Liver of Rats

Portions of liver tissues obtained from rats treated with FPE, ivermectin, levamisole and distilled water were kept at -4°C till. 1g of liver of each rat was crushed in 3 ml of phosphate buffer then centrifuged (3500 rpm for 15 min) and the supernatant was recovered in eppendorf tubes for biochemical analysis. The proportioning of the enzymes was carried out using a spectrophotometer of mark "MINDRAY BA-88A"; ALAT and ASAT kits were from Sigma-Aldrich (France).

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. Analysis of variance (ANOVA) using was used for the test of significant difference at 95% level ($P < 0.05$). T-test and Turkey's test at $P = 0.05$ was applied for mean separation. The LC₅₀ values were expressed as mean ± standard deviation of mean (mean of triplicates).

RESULTS

The FPE, levamisole and ivermectin were nematocidal in a time and concentration-dependent manner as shown in figures 1, 2 and 3. There was not mortality in 0.5% of DMSO in negative control.

Effect of Fruit Pulp Extract of *A. Digitata*, Levamisole, Ivermectin on *O. Ochengi* and *C. Elegans*.

Table 1: LC₅₀ values of the ethanolic FPE of *Adansonia digitata* and positive controls tested against *O. ochengi* and *C. elegans* (wild type and drug resistant strains) after 24, 48 and 72h exposure

LC ₅₀ (µg/mL) (95% FL) Khi-carré									
	24h			48h			72h		
Worms	Fruit pulp	Ivermectin	Levamisole	Fruit pulp	Ivermectin	Levamisole	Fruit pulp	Ivermectin	Levamisole
<i>O. ochengi</i>	36.01 ± 0.30 ^{abA} (20.03-50.06) 82.54***	0.18 ± 0.06 ^{ba} (0.15-1.44) 137.25***	nd	12.03 ± 1.22 ^{abB} (0.50-20.09) 53.30***	0.10 ± 0.07 ^{bb} (0.05-0.13) 45.97***	nd	5.05 ± 1.57 ^{ab} (0.10-10.09) 57.45***	0.07 ± 0.04 ^{bbB} (0.04-0.84) 47.89***	nd
<i>C. elegans</i> WT	82.08 ± 0.08 ^{abA} (21.01-177.30) 111.19*	2.75 ± 1.42 ^{ca} (1.54-3.56) 15.25 ^{ns}	5.82 ± 0.31 ^{ba} (4.97-6.51) 17.97 ^{ns}	60.02 ± 0.092 ^{abA} (17.01-78.20) 86.37***	2.45 ± 0.41 ^{ba} (0.95-3.59) 20.25*	3.94 ± 0.64 ^{bb} (2.76-4.73) 19.21 ^{ns}	49.01 ± 1.77 ^{abA} (15.52-57.32) 5.84***	2.23 ± 1.35 ^{ba} (1.22-3.88) 43.54***	3.56 ± 1.16 ^{bbB} (2.74-4.74) 38.80***
<i>C. elegans</i> CB211	209.03 ± 1.08 ^{abA} (127.04-293.01) 39.21***	nd		151.21 ± 1.19 ^{abB} (58.33-253.02) 95.62***	nd		95.61 ± 0.10 ^{abB} (44.04-149.21) 75.55***	nd	
<i>C. elegans</i> VC72 2	370.10 ± 0.08 ^{ba} (341.21-650.08) 57.44 ^{ns}		nd	224.0.3 ± 0.86 ^{abB} (138.09- 313.08) 26.32***		nd	210.06 ± 0.07 ^{abB} (98.14-590.02) 45.97***		nd

The LC₅₀ values are expressed as mean ± SDM of mean of triplicates. 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 determinate; FL: Fiducial Limit; ns: not significant; *p = 0. 05; ***p = 0.001.

Table 1 summarizes LC₅₀ values of ethanolic FPE of *A. digitata*, ivermectin and levamisole on *O. ochengi*. Ivermectin showed the highest efficiency on *O. ochengi* with the LC₅₀ values of. Compared to the LC₅₀ value of ivermectin and levamisole on *O. ochengi*, the ethanolic FPE of *A. digitata* showed the lowest nematocidal activity (p < 0.05) with a value of 5.05 ± 1.57 µg/mL. The LC₅₀ values of FPE of *A. digitata* and drugs (ivermectine and levamisole) against the wild type of *C.*

elegans at 24, 48 and 72 h. Ivermectin presented the highest LC₅₀ values compared to the FPE (p < 0.05).

Phytochemical Content of the Ethanolic Extract of *A. Digitata* Fruit Pulp

Determinations of tannins, phenolic acids, flavonoids and saponins content were conducted using photometrical quantification methods.

Table 2: Quantity (mg) of phytochemical compounds (tannins, flavonoids, saponins and phenolics) and percentage yield for each 100 grams of the ethanolic FPE of *A. digitata*

<i>A. digitata</i>	Tannins (mg/g GAE)	Flavonoids (mg rutin/100g)	Saponins (mg/g GAE)	Phenolic acids (mg/g GAE)
Fruit pulp (100g)	43.88 ± 2.57	0.07 ± 0.01	12.99 ± 0.10	21.28 ± 1.78

GAE: Gallic Acid Equivalent

Table 2 summarizes the amounts in equivalent mg of gallic acid per g of dry plant material (tannins, phenolic acids), in mg of rutin per 100 g of dry plant

material (flavonoids) and mg per g (saponins). The most abundant compounds were the tannins, phenolic acids and saponins. Flavonoids are also present but very low.

The resulting dry powder was weighed and expressed as percentage.

Toxicity Assays on Rats

The ethanolic FPE did not show any mortality at all doses tested after 72 h and up to 14 days. However, immediately after oral administration of FPE of *A. digitata*, some clinical signs of toxicity were observed like behavioral changes (locomotion, loss of appetite and sneezing) at 1750, 2000 and 5000 mg/kg.

The Effect of FPE on the Quantity of ALAT, ASAT in Function of Weight of Rats

ALAT and ASAT levels in rat liver (fig.4 and fig. 5) indicate that values are generally higher in male rats. The values ALAT and ASAT in positive group (CCL₄, ivermectin and levamisole) are generally highest than those of Negative control group.

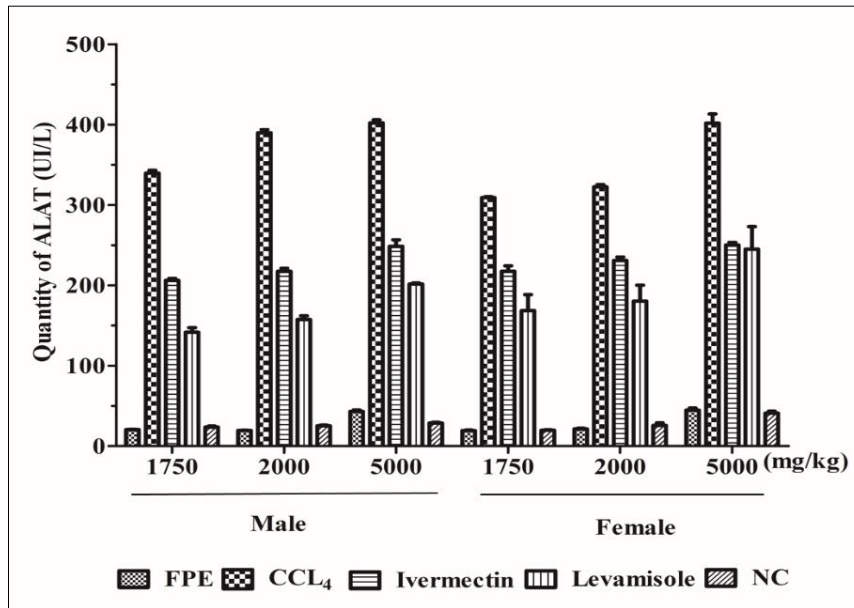


Figure 1: Effect of FPE, CCL₄, Ivermectine and Levamisole on the liver level of ALAT of rats. Values are expressed as mean ± SDM, Values are considered Significant if ($P < 0.05$) for all test. Mean= 18.63 ± 1.69, N=8; $t = -5.67$, $ddl = 7$, $p\text{-value} = 0.001$

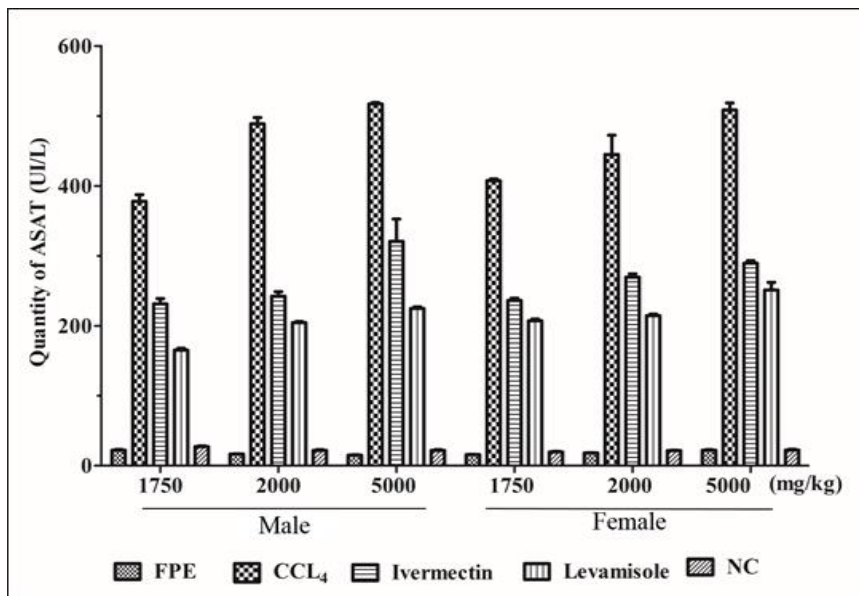


Figure 2: Effect of FPE, CCL₄, Ivermectine and Levamisole on the liver level of ASAT of rats. Values are expressed as mean ± SDM, Values are considered Significant if ($P < 0.05$) for all test. Mean= 18.63 ± 1.69, N=8; $t = -5.67$, $ddl = 7$, $p\text{-value} = 0.001$

DISCUSSION

Anthelmintic resistance, adverse effects and limited macrofilaricidal activities of the known

antifilarial drugs have stimulated the search for alternative treatments. The objective of this study was to evaluate *in vitro* the nematicidal activity of ethanolic FPE of *A. digitata* on parasite of cattle *O. ochengi* which is known to be a model for human onchocerciasis studies. For this study, we used wild type and mutant strains of the free-living model nematode *C. elegans* as well. Those mutants carry alleles conferring resistance towards the currently used antifilarial drugs ivermectin and levamisole. Those drugs were the positive control and DMSO ($\leq 1\%$ in each test) was included as negative control. The worms were subjected to increasing concentrations of ethanolic FPE or drugs and the mortality was determined after 24, 48 and 72h. The ethanolic FPE and drugs were remarkably active on the worms. There was not mortality observed in the negative control.

In general, at the maximum concentration of each of the used substances, 100 % mortality rate was registered at 72 h incubation. However, from 24 to 48 h weak concentration induced high mortality on both nematodes. The ethanolic FPE was active on *O. ochengi* with LC₅₀ values ranked from 5.05 to 36.01 $\mu\text{g/mL}$.

Several *in vitro* and *in vivo* studies have been carried out to determine the antiviral activity of various baobab plant parts. Ananil *et al.*, [45], investigated the antiviral activity of several plants against the herpes simplex, sindbis and polio viruses. Vimalanathan and Hudson [46], investigated the antiviral activity of *A. digitata* leaves, fruit-pulp and seed extracted with water, DMSO and methanol. The study was conducted using the minimum inhibitory concentration method against influenza virus, herpes simplex virus and the respiratory syncytial virus. Extract exhibited the most promising activity against the influenza virus with the MIC value ranging from 0.12 $\mu\text{g/ml}$ (DMSO) to 2.8 $\mu\text{g/ml}$ (water). In a recent other study, Masola *et al.*, [47], investigated the antimicrobial activity of baobab plant parts (stem and root barks) against Gram-positive bacteria, Gram-negative bacteria and yeast. In additional, Yagoub in 2008 [48], reported that the aqueous extract of *A. digitata* inhibited bacterial growth with the inhibition zone ranging from 20 to 30 mm depending on the concentration at which the sample was tested. Masola *et al.*, [47], mentioned that the antibacterial and antiviral activities of *A. digitata* could be attributed to the presence of tannins, phlobatannins, terpenoids and saponins in the stem bark.

The effect of FPE could also arise from its secondary metabolites content. In fact, the fruit pulp of *A. digitata* parts has showed effectiveness against numerous bacteria [49], and protozoa [28]. Parallel studies carried out with different plants [34-50], have demonstrated anti-Onchocerca and anthelmintic activities.

It is already established that filarial worms such as *O. ochengi* live with an endosymbiont (*Wolbachia*) [51], which could be targeted by that extract. The high activity on *O. ochengi* could come from the synergic effect of the diverse compound found in the FPE of *A. digitata* [52]. Several tannin-rich plant products showed effectiveness against *O. ochengi in vitro* [46, 47]. Elsewhere, the observed high efficiency of ivermectin is not observed at the same range *in vivo* because of the nodular wall which limits its activity on adult at low doses [53]. Nevertheless, purification and subsequent antifilarial screening of the pure compounds from the FPE will be required to determine its full potential.

For all the tested products *C. elegans* (wild type and mutants) was highly susceptible for the weak concentrations. This behavior could be an expression of an antagonist activity of a substance or compound of the FPE which inhibits the activity of the anthelmintic one. The effect of ethanolic FPE of *A. digitata* on *C. elegans* could be attributed to the activity of tannins as reported by Katiki *et al.*, [54], who reported that hydrolysable tannins-rich extracts or hydrolysable and condensed tannins-rich extracts are both significantly more lethal to adult of *C. elegans* than extracts containing only condensed tannins. The higher nematotoxicity of conventional drugs (figure 2A and 2B) which are pure substances denotes a difference with the ethanolic FPE in their mechanism of action. Ethanolic FPE as a substance cocktail may act on the same receptors as ivermectin and levamisole but with a lower specificity. A similar activity was observed with mutants to the FPE on *C. elegans* (figure 3) consequently, the FPE does not act with the same response as levamisole and ivermectin. In one hand, ivermectin is reported to bind to GluCl channels in the nerve or to nematode muscle cells with high affinity and increases the permeability to chloride ions across the cell membrane and consequently leads to cellular hyperpolarization and death of worms by paralysis [55]. In the other hand, levamisole is a nicotinic receptor agonist and causes hypercontraction of muscles and lethality due to prolonged activation of the excitatory nicotinic acetylcholine (nACh) receptors on body wall muscle [56]. Elsewhere, the activity of FPE on *C. elegans* (wild type and mutants) presented in figure 2C and figure 3 showed that the longer is the incubation time the stronger is the efficiency of the extract on the nematode.

Among the quantified phytochemical groups of FPE several have been reported as potential anthelmintic compounds. Tannins showed anthelmintic activity not only on *O. ochengi*, *C. elegans* [57], but on other nematodes too [58, 59]. Phenolic acids have been reported to induce mortality of *O. ochengi* and *C. elegans in vitro* [37].

The acute toxicity revealed that the ethanolic of the FPE is not toxic at all tested doses (1750, 2000 and 5000 mg/kg). The observed signs could be due to the

stressful situation of the rat related to their handling during the experiment. ASAT and ALAT are enzymes found in many tissues: liver, heart, kidney, muscles, intestines and they are commonly measured clinically as biomarkers for liver health [60]. In our study the transaminase levels of rats receiving extract are inferior to those of rats that received distilled water. The difference is not significant (p -value = 0.07) between the relative weight values of the organs of the rats that received the distilled water and those of the rats that received the extract. But the results of the ALAT and ASAT rates are relatively lower than the threshold values; this justifies that the doses of FPE of *A. digitata* used in our study would not be toxic to rats. Other study shown a significant protective effect of FPE of *A. digitata* extract against acetaminophen induced hepatotoxicity [61]. Hepatoprotective property of *A. digitata* extract could be explain by the capacity of b-sitosterol, b-amyrin palmitate, triterpenoids and ursolic acid presents in FPE to ameliorate lipid peroxidation by its scavenging activity of free radicals and increasing of the antioxidant defense system [62]. In addition, the protection and restoration of CCl₄-induced liver damage by FPE was also demonstrated by Al-Qarawi *et al.*, [63]. The consumption of FPE is undoubtedly an important factor in normal human resistance against liver damage due to endemic causes [64].

CONCLUSION

The current study was designed to investigate the nematocidal activity of the ethanolic FPE of *A. digitata*. The FPE was active against all worms tested and the highest activity recorded was against *O. ochengi*. It is known that biological activity of plants is attributed to the presence of phytochemical compounds. The phytochemical screening revealed the presence of flavonoids, tannins, saponins and phenolic acids. The acute toxicity of FPE tested *in vivo* on rats showed that the lethal dose was more than 5000 mg/kg following oral administration. The ALAT and ASAT rates of rats are relatively lower than the threshold values; this justifies that the doses of FPE of *A. digitata* used in our study would not be toxic to rats. Further studies on TLC, HPLC profile or finger-printing analysis, to isolate and purify active compounds present in FPE of *A. digitata* are needed.

Abbreviations

DMSO: dimethyl sulfoxide; O: *Onchocerca*, C: *Caenorhabditis*, FPE: fruit pulp extract, PBS: phosphate buffered saline; SDM: standard deviation of mean, 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, IRAD: Institute of Agricultural Research for Development.

Acknowledgements

We thank Prof. NDJONKA Dieudonné and Prof. EVA Liebau respectively from the University of Münster (Germany) and the University of Ngaoundere (Cameroon) for their all equipments and chemical materials used in this work.

Author's Contributions

NDOUWE TMH. Collected the plants, prepared the extracts performed the experiments, analyzed the results, and drafted the manuscript. KALMOBE J. and DIKTI V.J. co-performed the experiments, NDJONKA D. supervised the experiments, and corrected also the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors have declared no conflict of interest.

Ethics Approval: No applicable.

Consent to Participate: No applicable.

REFERENCES

- Burbelo PD, Leahy HP, Ladarola MJ, Nutman TB: A four-antigen mixture for rapid assessment of *Onchocerca volvulus* infection. *PLoS Negl Trop Dis* 2009, 3(5):ed 438.
- Schulz-Key H, Karam M: Periodic reproduction of *Onchocerca volvulus*. *Parasitology Today* 1986, 2:28486.
- Carme B and C Ripert: Onchocercose, encyclopédie médico-chirurgicale. *Elsevier, Paris* 1999, 8-514-A-40
- Kale OO: Onchocerciasis: the burden of the disease. *Ann Trop Med Parasit* 1998, 92(1):101115.
- OMS/APOC : Vue d'ensemble stratégique de l'avenir de la lutte contre l'onchocercose en Afrique, OMS/APOC/CAP, Ouagadougou, Aout 2006.
- Wogu MD, Okaka CE: Prevalence and socio-economic effects of onchocerciasis in Okpuje, Awan west local government area, Edo state, Nigeria. *International Journal of Biomedical and Health Sciences* 2008, 4(3): 113-119.
- World Health Organization: Onchocerciasis and its control. Report of a WHO Expert Committee on Onchocerciasis control. *World Health Organisation Technical Report Series* 1995, 852, 1-104, *World Health Organization, Geneva*.
- World Health Organization: Onchocerciasis and its control. Report of a WHO Expert Committee on Onchocerciasis control. *World Health Organisation Technical Report* 1995, *Geneva, Series: 852, 1-104*.
- WHO (The World Health Organization): Onchocerciasis (River blindness). *WHO* 2000, *Fact sheet* N°. 095.
- Tropical Disease Research (TDR): Onchocerciasis. *TDR Seventeenth Programme Report: Progress* 2003-2004, TDR/GEN/05.1: 44-49.
- De Sole G, Remme J: Onchocerciasis infection in children born during 14 years of *Simulium* control in

- West Africa. *Trans R Soc Trop Med Hyg* 1991, 85:38590.
12. Richards FO, Miri E, Meredith S *et al*: *Onchocerciasis Bull WHO* 1998, 76 (Suppl. 2):1479.
 13. Seketeli A, Adeoye G, Eyamba A *et al*: The achievements and challenges of the African Programme for Onchocerciasis Control (APOC). *Ann Trop Med Parasitol* 2002, 96 (Suppl. 1):1528.
 14. Lustigman S, McCarter JP: Ivermectin Resistance in *Onchocerca volvulus*: Toward a Genetic Basis. *PLoS Negl Trop Dis* 2007, 1: ed 76.
 15. Winnen M, Plaisier AP, Alley ES *et al*: Can ivermectin mass treatments eliminate onchocerciasis in Africa? *Bull WHO* 2002, 80: 38491.
 16. Cupp E. W. and M. S. Cupp: Short report: impact of ivermectin community-level treatments on elimination of adult *Onchocerca volvulus* when individuals receive multiple treatments per year, *The American Journal of Tropical Medicine and Hygiene* 2005, vol. 73, no. 6, pp. 11591161.
 17. Mccall JP, Townson H, Trees AJ: Morphometric differentiation of the *Onchocerca volvulus* and *O. ochengi* infective larvae. *Trans Roy Soc Med Hyg* 1992, 86:6365.
 18. Burglin, T.R., Lobos, E., Blaxter, M L: *Caenorhabditis elegans* as a model for parasitic nematodes. *Int. J. Parasitol* 1998, 28, 395411.
 19. Driscoll, M, Dean, E, Reilly, E, Bergholz, E, Chalfie, M: Genetic and Molecular Analysis of a *Caenorhabditis elegans* tubulin that conveys benzimidazole sensitivity. *J. Cell Biol* 1989, 109, 29933003.
 20. Dent J.A, Smith M.M, Vassilatis D.K, Avery L: The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A* 2000, 97, 26742679.
 21. Culetto E, Baylis, HA, Richmond JE, Jones, AK, Fleming JT, Squire, MD, Lewis J.A, Sattelle DB: The *Caenorhabditis elegans* unc-63 gene encodes a levamisole sensitive nicotinic acetylcholine. *J. Biol. Chem* 2004, 279, 4247642483.
 22. Tabuti JR, Dhillion SS, Lyea KA: Traditional medicine in Bulamogi County, Uganda, its practitioners, users and viability. *J Ethnopharmacol* 2003, 85:119-127.
 23. Harborne JB: *Phytochemical Methods*, Chapman and Hall Ltd. London 1973.
 24. WHO (The World Health Organization): Traditional medicine. *WHO fact sheet* 2008, N°134.
 25. Barrett D: From natural products to clinically useful antifungals. *Biochem. Bioph* 2002, Acta 1587:224-233.
 26. Abad MA, Ansuategui M, Bermejo P: Active antifungal from natural sources. *Arkivoc* 2007, pp. 116-145.
 27. Wickens GE, Lowe P: The baobabs: pachycauls of Africa. Madagascar and Australia, *Springer, UK*, 2008.
 28. Sidibe M, Williams JT: Baobab. *Adansonia digitata*. Fruits for the Future, 4. *International Centre for Underutilised Crops, Southampton, UK* 2002, p. 100.
 29. Chadaré F, Linnemann A, Hounhouigan J, Nout M, Van Boekel M: Baobab food products: a review on their composition and nutritional value. *Crit Rev Food Sci* 2009, 49:254-274.
 30. De Caluwé E, Halamová K, Van Damme P: Baobab (*Adansonia digitata* L.): a review of traditional uses, phytochemistry and pharmacology. In: Rodolfo H, Simon JE, Ho C.-T, (Eds.): African natural plant products: new discoveries and challenges in chemistry and quality. *Oxford University Press, USA* 2009, pp. 5184.
 31. Ramadan A, Harraz FM, and El Mougy SA: Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. *Fitoterapia* 1994, 65:418-422.
 32. Musila MF, Dossaji SF, Nguta JM, Lukhoba CW, Munyao JM. *In vivo* antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. *Journal of Ethnopharmacology* 2013; 146: 557-561.
 33. Van Staden J, Mulaudzi RB, Ndhkala AR, Kulkarni MG, Finnie J F. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. *Journal of Ethnopharmacology* 2011; 137: 330-337.
 34. Cho-Ngwa F, Abongwa M, Ngemenya MN, Nyongbela KD. Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca ochengi*. *BMC Complement Alternat. Med* 2010; 1062 (October 28). <http://dx.doi.org/10.1186/1472-6882> .
 35. Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achukwi D, Nukenine EN, Hensel A, Liebau E. *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and freeliving (*Caenorhabditis elegans*) nematodes. *J Helminthol* 2011; 85:304312.
 36. Ndjonka D, Ajonina-Ekoti I, Djafsia B, Luersen K, Abladam E, Liebau E: *Anogeissus leiocarpus* extract on the parasite nematode *Onchocerca ochengi* and on drug resistant mutant strains of the free-living nematode *Caenorhabditis elegans*. *Vet Parasitol* 2012; 190:136142.
 37. Johnson SJM, Castan L, Proudfoot DA, Barry and N. Christofi. Acute Toxicity of Linear Alkylbenzene to *Caenorhabditis elegans*. *Bull Environ Contam Toxicol* 2007; 79: 41-44.
 38. Thomas WK and Wilson AC. Mode and tempo of molecular evolution in the nematode *Caenorhabditis*: Cytochrome oxidase II and Calmodulin sequences. *Genetics* 1991; 128:269-79.
 39. Loveland BE, Johns TG, Mackay IR, Vaillant F, Wang ZX, Hertzog PJ. Validation of the MTT dye assay for enumeration of cells in proliferative and antiproliferative assays. *Biochem Int* 1992; 27:50110.

40. Schanderl SH, In. *Methods in Food Analysis. Academic Press New-York*, London 1970; PP: 709.
41. Folin O, Denis WA. colorimetric estimation of phenol and phenol and derivatives in urine. *J Biol Chem* 1915; 22: 305-308.
42. Wolfe K, WU X, and LIU RH. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* 2003; 51 (3): 609614.
43. Adedapo AA, Abatan MO, Olorunsogo O.O. Effects of some plants of spurge family on the haematological and biochemical parameters of rats. *Vet Arhiv* 2007; 77:29-38.
44. OECD. Reports of the international pre-validation and validation studies of the *in vitro* rodent alkaline comet assay for detection of *genotoxicity carcinogens*, Series on Testing and Assessment, Nos.195 and 196, OECD Publishing, Paris. 2014.
45. Ananil K, Hudson JB, De Souza C, Akpaganal K, Tower GHN, Amason, JT, Gbeassor M. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. *Pharmaceutical Biology* 2000; 38: 4045.
46. Vimalanathan S, Hudson JB. Multiple inflammatory and antiviral activities in *Adansonia digitata* (Baobab) leaves, fruits and seeds. *Journal of Medicinal Plants Research* 2009; 3: 576582.
47. Masola SN, Mosha, RD, Wambura PN. Assessment of antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae) (African Baobab). *African Journal of Biotechnology* 2009; 8: 50765083.
48. Yagoub SO. Anti-microbial activity of *Tamarindus indica* and *Adansonia digitata* extracts against *E. coli* isolated from urine and water specimens. *Research Journal of Microbiology* 2008; 3: 193197.
49. Manfredini S, Vertuani S, Buzzoni V. *Adansonia digitata*. Baobab farmacista. *L'integratore nutrizionale* 2002; 5: 2529.
50. 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.
51. Fenn K, Conlon C, Jones M, Quail MA, Holroyd NE, Parkhill J, et al. Phylogenetic Relationships of the *Wolbachia* of Nematodes and Arthropods. *PLoS Pathogens* 2006; 2(10): ed 94.
52. Nhukarume L, Chikwambi Z, Muchuweti M, Chipurura B. Phenolic content and antioxidant capacities of *Parinari curatelifolia*, *Strychnos spinosa* and *Adansonia digitata*. *Journal of Food Biochemistry* 2008; 34: 207221.
53. Catherine A, Outerbridge, and Susan Taylor M. *Oslerus osleri tracheobronchitis*: Treatment with ivermectin in 4 dogs, brief communication. *Can Vet J* 1998; 39: 238-240.
54. Katiki LM, Ferreira JF, Gonzalez JM, Zajac AM, Lindsay DS, Chagas AC, Amarante and AF: Anthelmintic effect of plant extracts containing condensed and hydrolysable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. *Vet Parasitol* 2012, 18: 218227.
55. Loughton DL, Lunt GG, Wolstenholme A J. Reporter gene constructs suggest that the *Caenorhabditis elegans* avermectin receptor beta-subunit is expressed solely in the pharynx. *J Exp Biol* 1997; 200: 15091514.
56. Ndjonka D, Mouraba A, Abakar A, Djafsia B, Ndouwe TMH. *In vivo* toxicity study and antifilarial activity of four plants from Nord- Cameroon. *European Journal of Medicinal Plants* 2017; 19 (3):1-12.
57. Hoste H, Brunet S, Paolini V, Bahuaud D, Chauveau S, Fouraste I, Lefrileux Y. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the Southern part of France. *Option Méditerranéennes* 2009; 431436.
58. Quijada J, Fryganas C, Ropiak HM, Ramsay A, Mueller-Harvey I, Hoste H. Anthelmintic Activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from small ruminants Are Influenced by Structural Features of Condensed Tannins. *J Agric Food Chem* 2015; Jul 22, 63: (28), 63:4654.
59. Chan-Pérez JI, Torres-Acosta JFJ, Sandoval-Castro CA, Hoste H, Castañeda-Ramírez GS, Vilarem G. *In vitro* susceptibility of ten *Haemonchus contortus* isolates from different geographical origins towards acetone: water extracts of two tannin rich plants. *Veterinary Parasitology* 2016 Feb; 15:5360.
60. Schuck S, Alain H: La douleur: Moyens et stratégies thérapeutiques. *La revue du praticien* 1997, 47:555-569.
61. Abeer Hanafy, HibahM. Aldawsari, JihanM. Badr, Amany K, Ibrahim and Seham El-Sayed Abdel-Hady: Evaluation of Hepatoprotective Activity of *Adansonia digitata* Extract on Acetaminophen-Induced Hepatotoxicity in Rats. Hindawi Publishing Corporation Evidence-Based *Complementary and Alternative Medicine* Volume 2016, Article ID 4579149, 7 pages <http://dx.doi.org/10.1155/2016/4579149>.
62. Ramesh D, Dennis TJ and MS Shingare: Constituents of *Adansonia digitata* root bark. *Fitoterapia* 1992, 63:278-279.
63. Al-Qarawi AA, Al-Damegh MA and El-Mougy SA: Hepatoprotective Influence of *Adansonia digitata* Pulp. *Journal of Herbs, Spices and Medicinal Plants* 2003, Vol. 10 (3).
64. Didibe M, Scheuring JF, Tembely D, Sidibe MM, Hofman P and Frigg M: Baobab-homegrown vitamin C for Africa. *Agroforestry Today* 1996, 8:13-15.