Review Article ISSN: 2349-2678

Contents lists available at www.ijpba.in
International Journal of Pharmaceutical and Biological Science Archive
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2019: 71.05
Volume 10 Issue 6; November-December; 2022; Page No. 72-87

Anti-*Onchocerca* Activity, Phytochemical Analysis and Toxicity Study of Ethanolic Extract of *Eucalyptus camaldulensis* on The Nematode Parasite *Onchocerca ochengi* Bwangamoi 1969

Bambe Raymond[1], Mando Ndou Bertrand[1], Amina Mamat[(1,3)], Koga Mang'dobara[(1,2)], Sokeng Dongmo Sélestin[1] , Ndjonka Dieudonné[1]*

[1]Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Po. Box 454 Ngaoundere, Cameroon.
[2]Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1 P.O. Box 812 Yaoundé, Cameroon.
[3]Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plant Studies, P.O. Box 13033 Yaoundé, Cameroon.

Conflicts of Interest: Nil

Corresponding author: Bambe Raymond

ABSTRACT

Background: The recent treatment of onchocerciasis remains the use of ivermectin which is the only drug against microfilaria and which resistant strains of veterinary importance are increasingly being revealed. In the aim to found the novel anti-filarial and alternative medicines, the anthelminthic activity of crude ethanolic extracts of leaves and bark of *Eucalyptus camaldulensis* were investigated on *Onchocerca ochengi*, a nematode parasite model for *Onchocerca volvulus*. The chemical compounds and toxicity of the extracts were also assessed.

Methods: *E. camaldulensis* leaves and bark Crudes extracts were prepared using hydro-ethanolic solvent (30 v distilled water and 70 v ethanol solvent). Anti-*Onchocerca* activity was determined in *vitro* on the parasitic nematodes *O. ochengi* and the viability of the worms was based on the standard method of dimethylthyazol (MTT) formazan test. Acute toxicity of the promising extracts was assessed in Wister rats. The phytochemical compounds of the ethanolic extracts were enrolled by colorimetric analysis using spectrophotometric (UV-visible).

Results: *E. camaldulensis* ethanolic leaf extract showed the highest anthelmintic activity against the adult male nematode *O. ochengi* with LC_{50} of 0.16 ± 0.01 mg/mL, as well as the ethanolic bark extract (LC_{50} = 0.19 ± 0.00 mg/mL) after 48 h of incubation. The quantitative phytochemical analysis revealed that *E. camaldulensis* ethanolic extracts showed highest quantity of polyphenols even in leaves (49.29±0.21 mg GAE/g) and barks (36.74±0.25 mg GAE/g). Flavonoids were abundant in the bark (14.56 ± 0.02 mg RE/g) and then 13.93 ± 0.09 mg RE/g for the leaves extract. Condense tannins were highly found in the ethanolic extract of bark (4.77 ± 0.07 mg CE/g) and that of leaves was 3.88 ± 0.07 mg CE/g. Saponins were found with the values of 0.66 ± 0.02 mg and 0.09 ± 0.04 mg respectively for leaves and bark ethanolic extracts of this plant. No critical behavioral changes and death were observed for the acute and sub-acute toxicity study.

Conclusion: This study revealed that *E. camaldulensis* ethanolic extracts were active against *O. ochengi* adult worms, demonstrating a possible new source of developing the anti-filarial phytomedicine for the treatment of human onchocerciasis.

Keyword: Anti-*Onchocerca*, *Onchocerca ochengi*, *Eucalyptus camaldulensis*, toxicity, phytochemical

Introduction

Human onchocerciasis or river blindness, caused by *Onchocerca volvulus*, remains a health problem for countries in Tropical Africa (99%), Latin America and the Arabian Peninsula (Yemen). This parasite is transmitted to human by a blackfly, *Simulium damnosum* during his blood meal [1]. The pathology of this diseases is manifested by the skin infection and ultimately ocular syndrome. According to the World Health Organization (WHO), about 21 million of people are affected by *O. volvulus,* with 240 million of the population at risk of contracting the disease [2]. The social burden of the onchocerciasis is the stigmatization and the long term of disability which lead to an unproductive population. This situation obliges the population to abandon their fertile area which is infected by the disease and consequently causes economic losses and lead to the retardation of country development [3, 4]. The main approaches employed in the control of onchocerciasis are the eradication of the vector involving spraying of insecticides, and the elimination of the parasite involving the mass treatment of the infected population with Ivermectin [5, 6]. This Ivermectin is the only microfilaricidal drug with limited effect on the adult worm [7]. Adverse effect of this treatment by including the lethal patient in the treatment area co-infected with *O. volvulus* and *Loa loa* [8] and the resistance of the parasite [7] limits the use of ivermectin. Thus, it is imperative to research a new, safe, efficacious and easily administrated macrofilaricidal drug against *O. vovulus*. It has been reported that plant are good sources of anthelminthic drugs and some anti-filaricidal activities of plant extracts have been studied [9]. *Eucalyptus amaldulensis* have been shown to express anti-bacterial activity on a range of Chemical Variability, Antifungal and Antioxidant activity [10, 11]. Other separate studies have revealed that *E. camaldulensis* have antioxidant, anti-inflammatory, antinociceptive and antimicrobial activities [12, 13]. However, anti-filarial activity of this plant has not been evaluated on the onchocerciasis worms in the best of our knowledge. In an attempt to contribute to the anti-*Onchocerca* lead development, we evaluated in this paper, the filaricidal properties of *E. camaldulensis* leaf and bark ethanolic crude extracts against *O. ochengi* adult male worms. Additionally, we report on the phytochemical and toxicity profile of the ethanolic extract of this plant. The anti-filarial activity was assessed against the adult worm of cow *O. ochengi*, a model of choice for *O. volvulus* drugs screens. These worms have as same vector *S. damnosum*, the same manifestation and phylogenetical lead [14, 15].

1. Material and Methods

1.1.Collection and identification of the plant

The leaves and bark of *E. camaldulensis* used in this study were collected in July 2017, from Dang village in Ngaoundere III Sub-Division Region of Adamawa, Cameroon. The plant was identified by a Botanist of the University of Ngaoundere and the voucher specimen was registered at the National Herbarium of Yaounde and assigned voucher number was N° 67480 HNC.

1.2.Preparation of crude extracts

The leaves and bark of plant collected were dried at a room temperature for three weeks, and then ground to fine powder. 100 g of powder plant organs were weighted and macerated in 1000 mL of ethanol-distilled water (70:30 v/v) for 48 hours in the air. The mixtures were filtered by centrifugation (3500×g, 10 min) and filter paper No 413 (VWR international, Darmstadt, Germany). The filtrate was concentrated by using a rotary evaporator under reduced pressure at 40° C and the concentrated filtrates were removed to dry at 40° C in the sauna. The dried crudes extracts were weighted and stored away from light at 4° C until using.

1.3.Preparation of the stock solutions for anthelminthic test

The stock solutions were prepared by weighing 300 µg of dry extracts and then introduced into the sterile falcon's tubes. 150 µL of DMSO (100 %) were added with 2850 µL of distilled

water for the final concentration of 100 µg/mL. For the conventional medicine (Ivermectin), the final concentration of 100 µg/mL was obtained by adding 300 µg of ivermectin in 3000 µL of distilled water. The mixtures were vigorously checked for homogenization (Vortex, Heidolph) and then stored at 4° C until usage.

1.4.Isolation and culture of *Onchocerca ochengi* adult worms

The isolation of *O. ochengi* adult worms was done according to the method described by Ndjonka *et al*. [16]. Briefly, the cattle's fresh pieces of umbilical skin with nodules were collected at the Ngaoundere slaughterhouse and sent to the laboratory for dissection. The skins were washed, drained and carefully sterilized with ethanol at 70 %, *O. ochengi* adult worms were extirpated out of the nodules using sterile bistoury razor. The mass containing adult males and females worms was temporary submerged in the physiologic medium [PBS: 4g NaCl; 0.1 g KCl; 0.72 g Na_2HPO_4; 0.12 g KH_2PO_4 for ½ liter] and the extirpated adult males of the worms were immediately submerged in Complete Culture Medium (CCM) [RPMI-1640: supplemented with L-glutamine (Sigma, Deisenhofen, Germany)].

1.5. *In vitro* *Onchocerca ochengi* test

The test was performed by using RPMI-1640 as Complete Culture Medium (CCM) for cultivation of *O. ochengi* as demonstrated by Borsboom *et al.* [17]. Dilution was prepared for each extract and the final concentrations obtained after dilution were 1.00, 0.75, 0.50, 0.40, 0.20 and 0.10 mg/mL. Negative control was prepared without the plant extract and the positive control was prepared with ivermectin (5 %). Each plant extract and CCM was added into vial container (plate for 96 wells) and worms were introduced in the mixture. With six worms for each concentration, one worm was introduced per well. The sensitivity of the parasite worm to the ethanolic extract of *E. camaldulensis* was determined biochemically (MTT/formazan) after incubation for 24 h and 48 h at 37° C at CO_2 incubator.

1.6. Worm mortality and LC_{50} determination

The biochemical reaction with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) made it possible to evaluate the mortality of the worms after the incubation times. In fact, the MTT 0.5 % was introduced into a 96-well plate to which the male worms of *O. ochengi* are found. After 30 min of incubation, the worms were observed under a binocular magnifying glass in order to identify the living and the dead worms. Yellow-colored worms were considered dead while purple-colored worms were considered─living. The mortality rate was determined by the ratio of dead to live worms. Each extract of the plant was tested in three independent determinations with their control group and the LC_{50} (lethal concentration of extract required to kill 50 % of worms) were determinates. Results are presented as mean value ± standard error of the mean (SEM). Negative control was treated with 0,5 % DMSO and ivermectin (5 mg/mL) was used as positive control.

1.7.Phytochemical assessment

1.7.1. Phytochemical screening

The standard method describes by N'Guessan *et al*. [18] was employed to carry out the phytochemicals compounds contents in the *E. camaldulensis* leaf and bark crudes extracts. Briefly, the presence of alkaloid was determined by using the Dragendorf reagent while the presence of flavonoid was determined by dissolving the ethanolic extract in methanol and 2-3 mL of concentrated HCl was added. In the mixture, the spatula full of magnesium turnings was added for the effervescence observation. The Lieberman Buchard reaction was used for triterpenes and sterols determination. For the tannin's determination, ferric chloride was used and the color change (mazarine, green or black) was observed. The saponins were determined by dissolving in water the ethanolic extract and shaking vigorously. Foaming in the solution was observed and considered as preliminary evidence. Reaction of quinone cycle in NH_4OH was employed as described by Bornträger and the violaceous red color was observed for the anthraquinone determination. The presence of polyphenols was gat out by using the ferric chloride method.

1.7.2. Quantitative phytochemical determination

1.7.2.1. Total Polyphenols determination

For 100 µL of each extract, 500 µL of Follin-Ciocalteu reagent was added to 400 µL of Sodium Carbonate (Na_2CO_3, 35 %). The mixture was vigorously shacked and then incubated at the obscure room for 10 min. Absorbance was read at a wavelength of 760 nm [19] with help of a spectrophotometer. The gallic acid game was evenly processed in the same condition and the results were expressed in milligram Gallic Acid Equivalent per gram of Plant Extract (mg GAE/g PE).

1.7.2.2. Total Flavonoid determination

Flavonoid compound of leaves and barks extracts of *E. camaldulensis* was determined following the protocol described by Kumaran et Karunakaran [19]. Subsequently, 1000 µL of ethanolic extract was added in 1000 µL of $AlCl_3$ (2 %) prepared in methanol. After vigorously agitated, solute was incubated at 30 min at the dark, and then the absorbance was read at 430 nm wavelength with the spectrophotometer. Rutin is the etalon substance for flavonoid determination and results are expressed in milligram Rutin Equivalent per gram of Plant Extract (mg Rut E/g PE).

1.7.2.3. Condensed tannin's determination

Following the vanillin method described by Wolfe *et al*. [20], condensed compound of tannins was carried out. The reagent solution was done by mixing equal volume of 37 % (v/v), 8 % (v/v) hydrochloric acid (HCl) and 4 % vanillin in methanol (m/v). To 1000 µL of vanillin reagent mixture, was added 200 µL of plant extract or Catechin and incubated in the obscure at 30° C for 20 min. The absorbance was red at the wavelength of 500 nm with the spectrophotometer. Total tannin contents in the plant extracts were calculated from Catechin etalon and expressed in milligram Catechin Equivalent per gram of Plant Extract (mg Cat E/g PE).

1.7.2.4. Saponins determination

The index foaming method was employed to quantify the saponins compound of the plant extract. In fact, 1000 µL of extract (0.1 mg/mL) was vigorously shacked and then allowed to rest about 5 second and the compound was determined according to the following formulas: Saponins (mg) = (0.432) × foaming height + 0.008 / mass of plant extract [19].

1.8. Acute toxicity study leaves extract of *Eucalyptus camaldulensis*.

Following the protocol described by the Organization of Economic Cooperation and Development (OECD) guideline 425, nulliparous and non-pregnant female's rats about 12 weeks old were used for this experiment [21]. A limit dose of 2000 mg/kg body weight of *E camaldulensis* ethanolic leaf extract was administered to these animals and distilled water was used as negative control. After the administration of the treated extract and negative control, a certain parameter such as somato-motor activity, skin changes, sleep, diarrhea and mortality for the first hours were recorded. Weight were also taken during the 14 days of observation period each week. The animals were sacrificed and the blood sample were collected for biochemical parameters as ALT, AST, UREA and creatine.

1.9. Subacute toxicity of ethanolic extract of *Eucalyptus camaldulensis*

The subacute toxicity of *E. camaldulensis* ethanolic leaves extract was conducted using 24 adults (female and male) Wistar albino rats (145-199 g). Animals were housed in standard cages (3 per cage), kept under ambient temperature and illuminated environment of 12:12 h dark/light cycle. The test was carried out according to the OEDC guideline No 407 [22]. Briefly, to the separated rate in each cage the different dose of extract was administered to each animal both group of male and female. Only the groups of control animals received the simple water. This operation was done every day for 28 days. Body weight was taken every 7 days and the behavioral animal were observed. At the last days of the toxicity studies, the rats were fasted overnight, sacrificed to perform hematological and biochemical analysis.

Statistical analysis

Tests were carried out in triplicate. The measured parameters during this study were expressed as Mean ± Standard Error of the Means (S.E.M). The data were compared by using analysis of variance (ANOVA) with the Bonferroni post-test to compare the replicates means. The difference was considered as significant when $P < 0.05$. The statistical analysis of the results was made using the GraphPad Prism version 5.03.

2. Results

2.1. Anthelminthic sensitivity test

The anthelmintic activities of *E. camaldulensis* leaf and bark ethanolic crude extract on adult male nematode *O. ochengi* worms were evaluated in terms of mortality in time of incubation and concentration. The results show that the increasing concentration of ivermectin as well as the ethanolic extracts of the leaves and bark of *E. camaldulensis* results in an increase in the adult male mortality *O. ochengi* in time and concentration in a dependent manner (fig 1). In fact, the ivermectin induced higher mortality of *O. ochengi* compared to the ethanolic extracts of *E. camaldulensis*. No mortality was recorded with the negative control. At the lowest concentration of 0.1 mg/mL, the leaf and bark extract induced respectively 33.33 % and 16.66 % of *O. ochengi* mortality rate after 48 h of incubation. The lethal concentrations required to kill 50 % incubated worms (LC_{50}) is presented in figure 2. After 24 and 48 h, LC_{50} of leaves extract were 0.40 ± 0.01 mg/mL and 0.16 ± 0.01 mg/mL respectively and that of bark were 0.42 ± 0.03 mg/mL and 0.19 ± 0.00 mg/mL respectively. For the positive control, LC_{50} values were 0.28 ± 0.05 mg/mL and 0.16 ± 0.01 mg/mL after 24 h and 48.

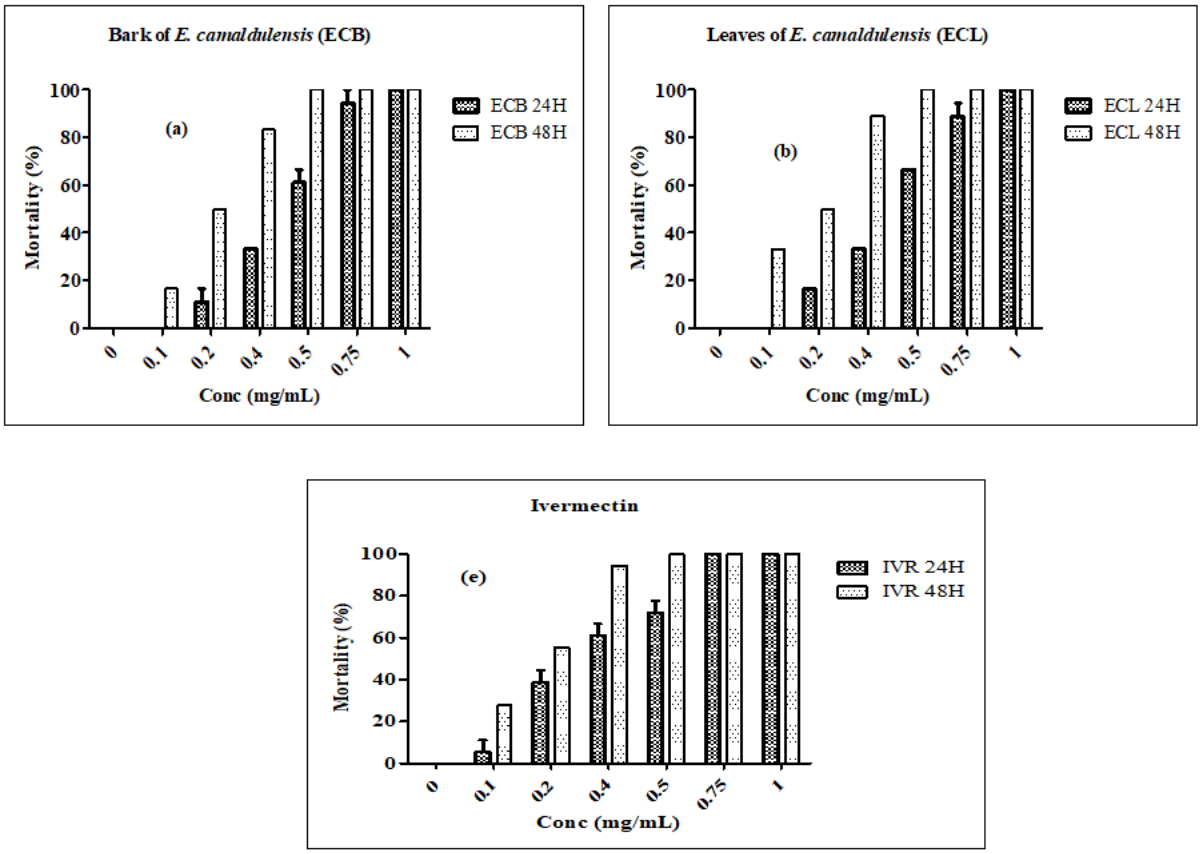

Figure 1: Anti-*Onchocera* activities of ethanolic extract of the parts of plants: (a) Ethanolic extract of leaves of *Eucalyptus camaldulensis* (ECL) and (b) Ethanolic extract of bark of *Eucalyptus camaldulensis* (ECB) after 24 h and 48 h of incubation; (c) Ivermectin (IVR). Ethanolic extracts act on *Onchocerca ochengi* in time and concentration depend manner. Data are mean ± SEM from three independent matching experiments.

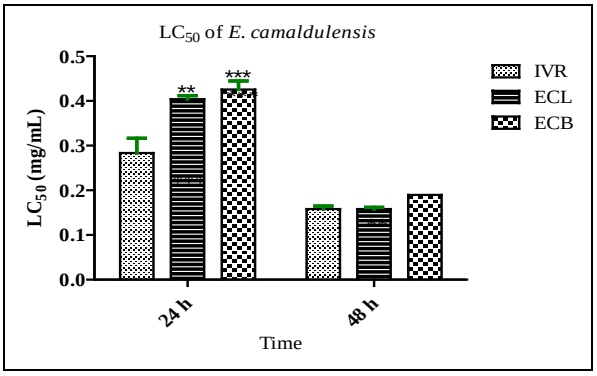

Figure 2: Lethal Concentration 50 (LC50) of ethanolic extract of the parts of plants: Ethanolic extract of leaves (ECL), bark (ECB) of *Eucalyptus camaldulensis* and Ivermectin (IVR) after 24 h and 48 h of incubation. The bars are mean ± SEM; n=3; ***p < 0.001 and **p < 0.01 are level of significance as compared to positive control test.

2.2. Phytochemical study of plant extracts

2.2.1. Phytochemical screening of the plant extracts.

The results of the phytochemical screening of the ethanolic extracts of leaves and bark of *E. camaldulensis* were studied, the results are presented in table 1. Negative indicated the absence while positive indicated the presence of particular metabolites. From these results, ethanolic extract of the leaves of *E. camaldulensis* revealed the presence of tannins, polyphenols, flavonoid, steroids, saponins and anthraquinons while the bark extract revealed the presence of tannins, polyphenols, flavonoids and terpenoids. The results of the quantitative determination of the chemical constituent of the extracts are presented in table 2. The ethanolic extracts of *E. camaldulensis* shows the highest quantity of polyphenols even in leaves (49.29 ± 0.21 mg GAE/g) and barks (36.74 ± 0.25 mg GAE/g). Flavonoids were abundant in the bark extracts of *E. camaldulensis* and then the leaves extract, with the respective values of 14.56 ± 0.02 mg RE/g and 13.93 ± 0.09 mg RE/g of extract. Condense tannins were highly found in the ethanolic bark extract of *E. camaldulensis* which registered 4.77 ± 0.07 mg CE/g of extract and that of leaves was 3.88 ± 0.07 mg CE/g. Saponins were found in the ethanolic extract of *E. camaldulensis* with the values of 0.66 ± 0.02 mg and 0.09±0.04 mg respectively for leaves and bark ethanolic extracts of this plant. However, there is no statistical difference observed between all the compounds quantified in all of the extract's plant.

Table 1: Phytochemical screening of the crudes ethanolic extracts of the leaves and bark of *Eucalyptus camaldulensis.*

Plant extracts	**Chemical compounds**							
	Tan	Alk	Pol	Sap	Fla	Ter	Ste	Ant
ECL	+	-	+	+	+	+	-	+
ECB	+	-	+	-	+	-	+	-

+ present; - absent. **ECL**: Ethanolic extract of leaves of *E. camaldulensis*; **ECB**: Ethanolic extract of bark of *E. camaldulensis*; **Tan**: Tannins; **Alk**: Alkaloids; **Pol**: Polyphenols; **Sap**: Saponins; **Fla**: Flavonoids; **Ter**: Terpenoids; **Ste**: Steroids; **Ant**: Anthraquinones.

Table 2: Quantity of chemical compounds of the crudes ethanolic extracts of the leaves and bark of *E. camaldulensis.*

Plants Extracts	**Chemical compounds**			
	TP (mgGAE/g)	TF (mg RE/g)	CT (mg CE/g)	TS (mg)
ECL	49.29 ± 0.21	13.93 ± 0.09	3.88 ± 0.07	0.66 ± 0.02
ECB	36.74 ± 0.25	14.56 ± 0.02	4.77 ± 0.07	0.09 ± 0.04

Values are mean ± SEM from three independent matching experiments; **ECL**: Ethanolic extracts of leaves; **ECB**: ethanolic extract of barks; **TP**: Total Polyphenols; **TF**: Total Flavonoids; **CT**: Condensed Tannins; **TS**: Total Saponins; **GAE**: Gallic Acid Equivalent; **RE**: Rutin Equivalent; **CE**: Catechin Equivalent.

2.3. Acute toxicity of active extracts

2.3.1. Mortality

The oral acute toxicity of the *E. camaldulensis* leaf crude extract was done in this study. The administration of the single dose of 2000 mg/kg body weight of extract shows no marked and adverse effect on the test rats. No death was recorded and the activity of all the animals was good during the 14 days of observation. So, the lethal dose (DL_{50}) was considered as to be up than 2000 mg/kg body weight for the ethanolic leaf extract of this plant. These results show that there was no marked of toxicity or physiological change on the rats.

2.3.2. Effect of the extract on the kidney and liver function indices

The effect of ethanolic extract of leaves of *E. camaldulensis* on liver and kidney function indices is presented in table 3. These results show that the oral administration of a single dose of extract for 14 days produce no significant difference in kidney and liver function indices in the treated groups and control group. Creatinine, urea, alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were not significantly different in control group and treated groups ($P > 0.05$).

Table 3: Biochemical parameters relating to blood of female rats (control, ethanolic leaves extract of *Eucalyptus camaldulensis*) after administration of a single dose of 2000 mg/kg.

Biochemical parameters	**Control group**	***E. camaldulensis* leaves**
AST (UI/L)	189.33 ± 34.50	271.67 ± 52.99^{ns}
ALT (UI/L)	168.33 ± 5.77	138.33 ± 43.11^{ns}
Urea (mg/mL)	43.22 ± 3,66	35.50 ± 4.65^{ns}
Creatinine (mg/mL)	0.90 ± 0.17	0.67 ± 0.12^{ns}

Values are mean ± S.E.M; n = 3; **ALT**: alanine aminotransferase; **AST**: Aspartate aminotransferase; ns: difference is not significant ($P > 0.05$) between treated groups and control.

2.4. Subacute toxicity of ethanolic extract of leaves of *Eucalyptus camaldulensis*

2.4.1. Effect of oral administration of *Eucalyptus camaldulensis* leaves extract on body weight and mortality

The effect of subacute toxicity of ethanolic extract of leaves of *E. camaldulensis* on the weight of females and males rats is reported in figure 3 and 4. At shows in these figures, Weights of females (fig 4) animals that received the repeated dose of *E. camaldulensis* ethanolic leaf extracts at the dose of 500 mg/kg shows no statistically significant difference ($p > 0.05$) compared to control group for all of the four (4) weeks. However, the difference was statistically considered ($p<0.05$) at week 2 and 3 at the doses of 250 mg/kg and 1000 mg/kg compared to control group. The significant difference ($p < 0.05$) was noted only in the doses of 250 mg/kg for all of the week's treatments in the groups of males which received the ethanolic leaf extract compared to the control group (fig 4). No lethality was recorded for all of the dose during 4 weeks (28 days) of treatment.

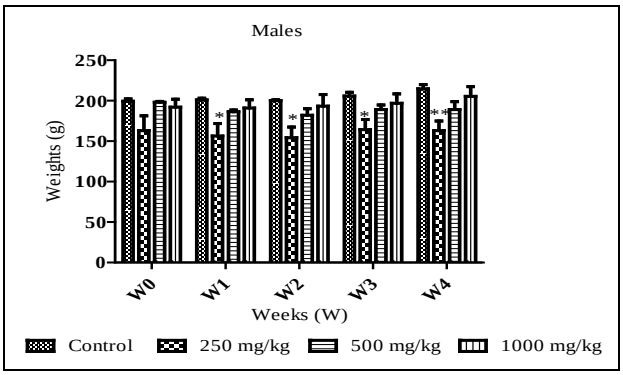

Figure 3 : Evolution of body weights of males' rats control group and groups receiving–ethanolic leaf extract of *Eucalyptus camaldulensis* administered by oral route for 28 days. Level of statistical significant difference are *p < 0.05; **p < 0.01.

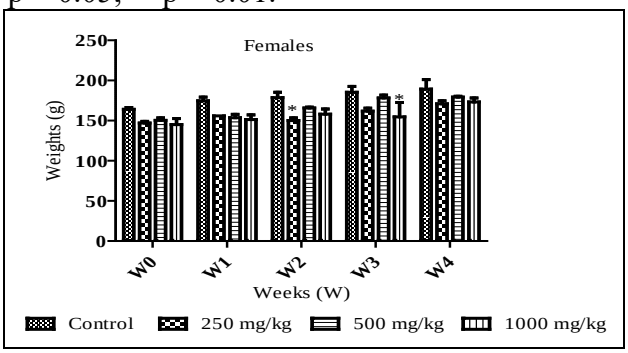

Figure 4 : Evolution of body weights of females' rats control groups and groups receiving ethanolic leaf extract of *Eucalyptus camaldulensis* administered by oral route for 28 days. Level of statistical significant difference is *p < 0.05.

2.4.2. Effect of *Eucalyptus camaldulensis* ethanolic leaf extract on the biochemical parameters of rats after 28 days of treatments

In the table 4, we recorded the results of biochemical blood parameters of the animals for ALT, AST, urea, creatinine and albumin. This table shows that there was not statistical significance difference between treated groups and control group in female rats. According to the groups of male rats treated with *E. camaldulensis* leaf extract, ALT significantly increased at the dose of 250 mg/mL (P < 0.001), while AST significantly increased at the dose of 1000 mg/kg (P < 0.01) compared to the control. Regarding to urea, creatinine and albumin levels, no statistical difference was observed in the treated groups compared to the control group (p > 0.05).

Table 4: Biochemical parameters relating to blood of animals control and ethanolic leaves extract of *Eucalyptus camaldulensis* after 28 days of administration of the repeated dose toxicity study.

Biochemical parameters	Sex	Control	250 mg/mL	500 mg/mL	1000 mg/mL
ALT (UI/L)	M	67.33 ± 17.47	147.00 ± 22.87***	88.00 ± 5.00	91.00 ± 12.12
	F	106.33 ± 56.92	62.33 ± 9.07	79.33 ± 3.51	75.33 ± 0.58
AST (UI/L)	M	156.67 ± 67.33	191.33 ± 6.51	186.67 ± 5.51	206.67 ± 25.32*
	F	227.33 ± 107.95	208.00 ± 38.15	207.67 ± 7.51	215.00 ± 25.00
Creatinine (mg/mL)	M	0.67 ± 0.12	0.73 ± 0.05	0.67 ± 0.06	0.70 ± 0.10
	F	0.57 ± 0.06	0.63 ± 0.06	0.77 ± 0.06	0.67 ± 0.06
Urea (mg/mL)	M	17.40 ± 2.34	22.10 ± 3.50	17.10 ± 0.90	18.70 ± 1.40
	F	17.40 ±1.01	16.97 ± 0.70	21.00 ± 0.40	19.30 ± 0.40
Albumin (g/L)	M	34.80 ± 2.94	33.33 ± 3.00	35.5 ± 3.30	34.10 ± 2.25
	F	36.43 ± 3.93	33.90 ± 1.32	37.83 ± 0.45	38.57 ± 2.66

The ethanolic extract of *E. camaldulensis* was given daily by oral route to groups of rats (n=6) at the doses: 250 mg/kg, 500 mg/kg and 1000 mg/kg for 28 days. Biochemical parameters were measured after the last day of treatment. The data are expressed as mean ±S.E.M. Level of statistical significant difference is: *P< 0.05; ***P<0.001. M: Male; F: Female.

2.4.3. Effect of ethanolic extract of leaves of *Eucalyptus camaldulensis* on haematological parameters of rats after 28 days of treatments

The effect of *E. camaldulensis* leaf extract on the haematological parameters of the treated groups and control groups of rats is shown in Table 5. In this table, there were no significant differences in White Blood Cells, Red blood cells and Haematocrit during the 28 days of treatment period (P > 0.05) at all the tested doses for both sexes (Table 5). But there was a significant increase in the values of Platelets in the experimental group which received the doses of 250 mg/kg and 500 mg/kg as compared to the control groups for females (P < 0.001) and decreased at the dose of 1000 mg/kg. In male treated groups, the platelets value were decreased at the group treated by the dose of 250 mg/kg, while this value was passed from 665 ± 74.00 (× 10/L) in the control group to 768.50 ± 46.50 (×10/L) at the dose of 500 mg/kg. The difference was not statistically considered at the dose of 1000 mg/kg compared to the control group.

Table 5: Haematological parameters of rodents treated with *Eucalyptus camaldulensis* ethanolic leaf extract after 28 days of treatment

Haematological Parameters	Sex	Treated groups			
		Control	250 mg/mL	500 mg/mL	1000 mg/mL
Platelets (10/L)	M	665.00±74.00	621.67 ± 57.37	768.5 ± 46.5**	699.33± 65.96
	F	498.00 ±161.06	660.67 ± 117.75***	832.67±112.50***	679.00±80.50***
White Blood Cells (10/L)	M	8.00 ± 2.79	6.86 ± 0.85	7.75 ± 1.85	7 ± 2.32
	F	8.83 ± 4.57	6.00 ± 1.65	6.65 ± 1.05	7.15 ± 0.05
Red blood Cells (10/L)	M	7.41 ± 0.01	7.13 ± 0.51	6.63 ± 0.26	7.13 ± 0.67
	F	6.92 ± 0.43	6.56 ± 0.65	7.06 ± 0.17	7.47 ± 0.02
Haematocrits (%)	M	44.78 ± 3.20	38.73 ± 0.76	43.65 ± 1.45	45.43 ± 2.27
	F	43.17 ± 1.70	45.90 ± 0.85	44.6 ± 2.9	44.5 ± 3.3

The *E. camaldulensis* ethanolic leaf extract was given daily by oral route to groups of rats (n=6) at the doses: 250 mg/kg, 500 mg/kg and 1000 mg/kg for 28 days. Haematological parameters were measured after the last day of treatment. The values are expressed as mean ± S.E.M. Level of statistical significant difference is: ***P<0.001; *P< 0.05; **P<0.01. M: Male; F: Female.

2.4.4. Effect of *Eucalyptus camaldulensis* ethanolic leaf extract on the organs of rats after 28 days of treatments

Table 6 presents the results of average organ weight of male and female rats. In females treated groups, no statistical difference (P > 0.05) was observed between the relative weight of liver, kidney, spleen, lungs and heart of the different groups that received the different doses of *E. camaldulensis* ethanolic leaf extract compared to the control group. In the male treated groups, no significant difference (P > 0.05) was observed between the relative weight of kidney, spleen and the heart of the different treated groups and the control group at all doses. However, in the male group treated, for the liver gain weight (3.73 ± 0.23 g) at the dose of 1000 mg/kg, a significant increase in relative weight were observed (P < 0.01) compared to the control group (3.25 ± 0.05).

Table 6: Weights of organs from rats in control groups and groups receiving ethanolic extract leaves of *Eucalytus camaldulensis* administered by oral route for 28 days

Organs	Sex	Control groups	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	**F**	3.33 ± 0.14	4.03 ± 0,19	3.30 ± 0.20	3.94 ± 0.17
	M	3.25 ± 0.05	3.34 ± 1,13	3.65 ± 0.17	3.73 ± 0.23 *
Kidney	**F**	0.80 ± 0.06	0.79 ± 0,05	0.82 ± 0.01	0.71 ± 0.02
	M	0.70 ± 0.01	0.85 ± 0,20	0.72 ± 0.03	0.60 ± 0.13
Spleen	**F**	0.41 ± 0.05	0.43 ± 0,12	0.35 ± 0.01	0.35 ± 0.02
	M	0.25 ± 0.03	0.34 ± 0,11	0.51 ± 0.14	0.40 ± 0.11
Lungs	**F**	1.25 ± 0.21	1.14 ± 0,22	1.16 ± 0.03	1.23 ± 0.15
	M	1.47 ± 0.20	1.32 ± 0,10	1.32 ± 0.52	1.17 ± 0.05
Heart	**F**	0.34 ± 0.04	0.35 ± 0,02	0.37 ± 0.00	0.39 ± 0.00
	M	0.34 ± 0.03	0.43 ± 0,09	0.40 ± 0.03	0.35 ± 0.02

The ethanolic extract of *E. camaldulensis* was given daily by oral route to groups of rats (n=6) at the doses: 250 mg/kg, 500 mg/kg and 1000 mg/kg for 28 days. Relative weight organs were measured after the last day of treatment. The values are expressed as mean ± S.E.M. Level of statistical significant difference is: *P<0.05; **P<0.01. F: Female; M: Male.

Discussion

The main objective of the present study was to analyse the phytochemical compounds, evaluate the *in vitro* anti-*Onchocerca* activities of the ethanolic extract of leaves and bark of *E. camaldulensis* on the parasite nematode *O. ochengi*, a model organism of study of the Neglected Tropical Disease which is a human onchocerciasis and study the toxicity of the promising extract on rats.

In this study, some phytochemicals compounds were quantified as show in table 2. It appears from this table that, the high quantity of chemical compound were polyphenols which is found in majority in *E. camalduensis* ethanolic leaf extract followed by that of bark. Flavonoids are abundant in both leaves and bark extract. Tannins are present in majority in bark and saponins is found in leaves. It is know that by adding water to pure ethanol up to 70 %, the polarity of solvent is increased [23]. According to other researchers [24, 25] the probability effect of phytochemical compounds is related to the ability of the nematodes to make the complex and create interaction with the parasite surface proteins disrupting essential functions such as reproduction and nutrition.

The ethanolic extract of leaves and bark of *E. camaldulensis* inhibited the survival of the parasite nematode *O. ochengi* in terms of concentration and time as show in figure 1. According to Ndjonka *et al*. [16] considering the degree of inhibition, an extract is considered to be more effective (LC_{50} < 2.5 mg/mL), moderate (2.5 mg/mL < LC_{50} < 4mg/mL) or not having anthelmintic activity (LC_{50} > 4 mg/mL).

The leaves and bark extracts of this plant displayed the highest anthelmintic activity with LC_{50} values of 0.16 ± 0.01 mg/mL and 0.19 ± 0.00 mg/mL respectively after 48 h of incubation. Compared to ivermectin, all our extracts were significantly different regardless of the LC_{50} at 24 of incubation. At 48 h of incubation, the difference was no significant between ivermectin and the parts of *E. camaldulensis*. These results show that the extracts inflame the survival of the *O. ochengi* worms as time and concentration dependant. Similar results are reported by Ndjonka *et al*. [16] whom focused on the *in vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*O. ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. Theses researchers concluded that worm mortality does not vary only with concentration, but also with the part of plant and time. Their work on the extract of *Anogeissus leiocarpus* have also gave the same results [26], as well as those obtained by Koga *et al*. [27] who studied the

anthelmintic activity of the methanolic extract of *Canarium schweinfurthii* depending on time and concentration. These cannot be explained only by the time the active substance takes to bind with cells of worms, but also by the quantity of these active substances found in the extract, thus making it possible to paralyze and kill *O. ochengi*. Several studies report that anthelmintic activity of plant extracts is due to secondary molecules [9, 27].

The secondary compounds such as flavonoids, tannins, saponins and polyphenols in the plant extract can be responsible for the preliminary explanation of its nematicidal activities. Synthetic anthelmintic exert their effect in various ways, and target parasite's cell membrane resulting to the paralysis followed by the death of the worms. So as ivermectin drug, this macro-cyclic molecule acts on the *O. volvulus* ling-gated channel which is the gated glutamate.

In the cell, ivermectin reacts in many different ways. The chloro-dependant channel of glutamate (Glu-Cl), on the cell member of neuro muscular of nematodes and arthropods has been his major site of action. The binding of ivermectin on the glutamate receptor train a Cl- ion flux on the cell and the hyperpolarisation of this one. In the nematodes, the Glu-Cl are particularly present at the level of the pharynx muscular and the ivermectin inhibit the pharyngeal pumping activity, and then the ingestion of nutriment. Moreover, ivermectin can act also on the GABA receptor. The lack of Glu-Cl on trematode and cestode would explain the inefficiency of the ivermectin on this group of helminths.

The likely hypothesis of effect of the ethanolic extracts at the level of Glu-Cl and GABA receptor complex is confirmed by the results of anthelminthic test *in vitro* done on the adult's male of *O. ochengi* worms by ivermectin, which is the compound acting on the Glu-Cl and GABAergic neurotransmission. The presence of certain bioactive substances such as tannins, polyphenols, flavonoids and saponins of *E. camaldulensis* ethanolic extracts could explain in part the anthelminthic activity of the plant. The tannins (ellagic, gentilic and gallic acids) [28, 29] and flavonoid (C-O-C Flavonoid Dimmer) [30] compound have been reported to increase the mortality rate of *O. ochengi* in term of concentration and time.

Toxicity study on animals will be critical for positive judgment on the safety of plant extract if they are found to have adequate potential for development into pharmacological compounds [31]. Hence, in the present study, acute oral administration of single doses of the ethanolic extract leaves of *E. camaldulensis* up to 2000 mg/kg neither caused death nor changed the behaviour of the rats. Therefore, the LD_{50} for leaves extracts was above 2000 mg/kg. According to Organization for Economic Development (OECD) criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD_{50} > 2000-5000 mg/kg are categorised as unclassified or category 5 [32]. This value puts the ethanolic leaf extracts of *E. camaldulensis* under GHS Category, implying that extract is relatively low acute toxicity.

Analysis of biochemical blood parameters is important in the evaluation of risk associated with test compounds [33]. Transaminase such as AST and ALT are known as good indicators of liver function and used as biomarkers to determine the probably toxicity of drugs [34]. There were no changes in AST and ALT level, which reveal that the ethanolic extract of leaves does not affect the liver function. The normal values of kidney parameters (urea and creatinine) suggest that the extract did not cause any damage to the kidney. In this study, we can conclude that the ethanolic extract of leaves of *E. camaldulensis* is not toxic on animals and can be provided as source of anti-helminthic drugs. Acute toxicity data are usually of limited clinical application. Therefore, sub-acute toxicity study was carried out.

Monitoring of the weight change showed the presence of a significant loss in weight in the treated groups (250 mg/kg) of male compared with the control group. In the female treated groups, a significant loss in weight was observed at week 2 at the dose of 250 mg/kg, and the dose of 500 mg/kg at week 3. Partially, other authors have also shown the effect of oral

administration of plant extract on the body weight of animals [35, 36]. Weight change is used as a general indicator of adverse effects of chemical compounds [37]. Thus, the weight loss is correlated to the physiological state of the animal can be explained not only by the alteration of the metabolism of the animals, but also by anorexia [38]. Loss of appetite often lead to weight loss due to disturbances in the metabolism of lipids, proteins or carbohydrates [39, 40].

According to relative organ weight, no significant difference was observed in liver, kidney, spleen, lung and heart in the female groups treated with ethanolic leaf extract *E. camaldulensis*. Similarly, no significant change was observed in the group of males treated with ethanolic extract of *E. camaldulensis*, except in the relative weight of the liver and lung. The relative weight was significantly increased in these organs. Relative organ weight is considered as a relatively sensitive indicator in toxicity studies [41]. The role of kidney is to purify blood and eliminate waste, while the role of the hepatocytes is to neutralize toxins or detoxify the organism [42]. Kidney dysfunction can be assessed by concurrent measurement of urea creatinine and uric acid [35]. In this study, change in plasma urea and creatinine in blood of treated animals in the both male and female, which receiving the ethanolic extract of leaves of *E. camaldulensis*, showed no significant difference indicating a normal renal function. This result is in agreement with Azza *et al*. [41] whom found that ethanolic leaves extract of *E. camaldulensis* from Sudan does not damage kidney function. The increasing level of ALT and AST in the blood are due to release following damage to liver cells [43]. AST is found primarily in the kidney, the red blood cells and cardiac and skeletal muscle while ALT is found in the liver and is the most sensitive for the liver cells damage. AST is not specific to liver as ALT. In this study, the repeated administration of the ethanolic extract of *E. camaldulensis* leaves resulted in a highly significant increase in levels of the enzyme ALT only at the lowest dose (250 mg/kg) in male treated rats. But the highest doses are not increase in the level of ALT. In most case, liver enzymes are elevated mildly and temporarily. Most of the time, elevated liver enzymes don't signal a chronic serious liver injury. Indeed, an increase in the membrane permeability of the hepatocyte cells can lead to the flow of these enzymes into the bloodstream and therefore an increase in their serum level [35]. According to study done by Kabiru *et al*. [44] on the methanolic extract of *E. camaldulensis* leaves, the lower level of ALT as well as highest in the bloodstream is not increase. These researchers made the histological section of liver and found that the liver cells were not affected. Same result was found with ethanolic extract of leaves of *E. camadulensis* in this study with doses of 500 mg/mL and 1000 mg/mL in male treated groups. No difference was noted between the female groups treated compared to control group. This suggest that the ethanolic extract of *E. camaldulensis* is not damage the liver function.

The blood indices are often use as an indicator pathological and physiological of the body and significant change imply that the administrated of chemical compound or vegetal substance is either protective or toxic to the haematopoietic tissue. Finding of our research report that administration of ethanolic extract of *E. camaldulensis* leaves in male groups for the period of 28 days produced no significant different in all blood parameters except an increase in means platelets volumes at the dose of 500 mg/kg. According to the female groups treated with the doses of ethanolic extract of *E. camaldulensis*, the non-significant effect of the extract on total red blood cells, total white blood cells and Hb indicates that the extract does not affect the erythropoiesis, or morphology of the red blood cells [45]. These results are agree with Azza *et al*. [41] which found non-significant level of blood parameters in rats treated with ethanolic extract of leaves of *E. camaldulensis*. The means values of platelets were highly increase at all of the dose in this test. The increase in platelets in blood suggest the possible haemostatic disorder and eventually lead to thromboembolic diseases [46]. This increase of platelets in this study may be due to the immunostimulatory effects of the chemical compounds in the extract and also

indicates that this plant could possess antianemia property. Our result is similar to those of Ntchapda *et al*. [47] and Mando *et al*. [36], which showed that oral administration of *Ficus glumosa* as well as *Gmelina arborea* induced the significant increase in the level of platelets in rats.

Conclusion

The current studies have provided the first indication that ethanolic leaf extract of *E. camaldulensis* possess anti-helminthic activity. According to the two extracts (leaf and bark) of the plant in this study, the phytochemical compound responsible for anti-*Onchocerca* activity may be well placed in the ethanolic leaf extract than in bark extract. This finding suggests that the extract of *E. camalduelensis* can serve as the potential source of a new anti-filaricidal compound for the treatment of human onchocerciasis and other helminths infections.

References

1. OMS. Elimination of human onchocerciasis: progress report 2020, Weekly Epidemiological Record.2021; 46:557-667.

2. Luu L, Bah GS, OkahNnane NH, Hartley CS, Glover AF, Walsh TR, Lian L-Y. Zhan B, Bottazzi ME, Abraham D, Petrovsky N, Bayang N, Tangwa B, Ayiseh RB, Mbah GE, Ekale DD, Tanya VN, Lustigman S, Makepeace BL, Graham-Brown J. Co-administration of Adjuvanted Recombinant Ov-103 and Ov-RAL-2 Vaccines Confer Protection against Natural Challenge in A Bovine *Onchocerca ochengi* Infection Model of Human Onchocerciasis. *Vaccines*. 2022; 10:861. https://doi.org/10.3390/ vaccines10060861.

3. Philippon B. Etude de la transmission de *Onchocerca volvulus* (Leuckart, 1893) (Nematoda, Onchocercidae) par *Simulium damnosum* Theobald, 1903 (Diptera, Simulidae) en Afrique tropicale. *Travaux et documents de l 'O.R.S.T.O.M.* 1977 ; 63 : 308 p.

4. OMS. Comment reconnaître les maladies tropicales négligées selon les altérations de la peau : guide de formation à l'usage du personnel sanitaire de premières lignes [Recognizing neglected tropical diseases through changes on the skin : a training guide for front-line health workers]. Genève : Organisation mondiale de la Santé. 2018. Licence :CC BY-NC-SA 3.0 IGO.

5. Diawara L, Traore MO, Badji A, Bissan Y, Doumbia K, Goita SF, Konate´ L, Mounkoro K, Sarr MD, Seck AF, Toe´ L, Toure´e S, Remme JHF. Feasibility of Onchocerciasis Elimination with Ivermectin Treatment in Endemic Foci in Africa: First Evidence from Studies in Mali and Senegal. *PLoS Negl Trop Dis*. 2009; 3(7): 497. doi:10.1371/journal.pntd.0000497.

6. Samje M, Metuge J, Mbah J, Nguesson B, Cho-Ngwa F. In vitro anti-*Onchocerca ochengi* activities of extracts and chromatographic fractions of *Craterispermum laurinum* and *Morinda lucida*. *BMC Compl. Altern*. Med. 2014; 14: 325.

7. Osei-Atweneboana MY, Jeffrey KLE, Boakye AD, Gyapong OJ, Prichard KR. Prevalence and intensity of Onchocerca volvulus infection and efficacy of ivermectin in endemic communities in Ghana: a two-phase epidemiological study. *Lancet*. 2007; 369:2021-2029.

8. Takougang I, Muteba D. Encephalopathy Related to Ivermectin Treatment of Onchocerciasis in *Loa loa* Endemic Areas: Operational Considerations, Miscellanea on Encephalopathies, Dr. Radu Tanasescu (Ed.).2012. ISBN: 978-953-51-0499-5.

9. Ndjonka D, Djafsia B, Liebau E. Review on medicinal plants and natural compounds as anti-*Onchocerca* agents. *Parasitology Research*. 2018; 17p. https://doi.org/10.1007/s00436-018-6003-7.

10. Barra A, Coroneo V, Dessi S, Cabras P, Angioni A. Chemical Variability, Antifungal and Antioxidant Activity of *Eucalyptus camaldulensis* Essential Oil from Sardinia. *Natural Product Communications*. 2010 ; 5 (2):329-335.

11. Sliti S, Ayadi S, Kachouri F, Khouja MA, Abderrabba M, Bouzouita N. Leaf essential oils chemical composition, antibacterial and antioxidant activities of *Eucalyptus camaldulensis* and *E. rudis* from korbous (Tunisia) J. Mater. Environ. Sci. 2015; 6 (3):743-748. ISSN: 2028-2508 CODEN: JMESCN.

12. Traoré N, Bouaré S, Sidibe L, Somboro AA, Fofana B, Tangara O, Diallo D, Chalchat JC, Figuiredo G. Antimicrobial activity of essential oils of *Eucalyptus camaldulensis* from Mali. *Asian J. Plant Sci. Res*.2014; 4(4):69-73.

13. Olawore NO, Ololade ZS. *Eucalyptus camaldulensis* var. nancy and *Eucalyptus camaldulensis* var. petford Seed Essential Oils: Phytochemicals and Therapeutic Potentials. *Chem Sci* J. 2017; 8:148. doi: 10.4172/2150-3494.1000148.

14. Hoch B, Wahl G, Enyong P, Luder CK, Harnett W, Renz A. Serological recognition of specific in cross reactive antigens of *Onchocerca ochengi Onchocerca volvulus* by infected cattle and humans. *Tropical Medicine in Parasitology*.1992; 43:206-207.

15. Wahl G, Achukwi DM, Mbah D, Dawa O, Renz A. Bovine onchocerciasis in North Cameroon. *Veterinary Parasitology*. 1994; 52: 297-311.

16. Ndjonka D, Agyare C, Luersen K, Djafsia B, Liebau E. *In vitro* activity of Camerounian and Ghanian medicinal plants on parasitic (*Onchocerca ochengi*) and free living neematode (*Caenorhabditis elegans*). *Journal of Helminthology*. 2011; 85:304-312.

17. Borsboom GJ, Boatin BA, Ngelkerke NJ, Agoua H, Akpoboua KL, Alley EW, Bissan Y, Renz A, Yameyogo L, Remme JH, Habbema JD. Impact of ivermectin on onchocerciacis transmission: assessing an empirical evidence that repeated Ivermectin treatements may lead to elimination/elimination in West-Africa. *Filaria Journal*.2003; 2:8.

18. N'Guessan K, Kadja B, Zirihi GN, Traoré D et Aké-Assi L. Phytochimic Screening of some medicinal plants of the Ivoryan (used in Krobou country, Côte -d'Ivoire). *Science of Nature*. 2009; 6:1-15.

19. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem*. 2006; 97:109-14.

20. Wolfe K, Wu X, Liu RH, 2003. Antioxidant activity of apple peels. *J Agric Food Chem*. 2003; 51:609-614.

21. OECD. Organization for Economic Cooperation and Development Guidelines for the testing of chemicals. 2008; No 425.

22. OECD. Organisation for Economic Cooperation and development Guideline for testing of Chemicals. 2008; No 407.

23. Bimakr M. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. *Food Bioprod Process*. 2010; 1-6.

24. Martin RJ. Mode of action of anthelmintic drugs. *Vet. J*. 1997; 154:11-34.

25. Thompson DP, Geary TG. The structure and function of helminth surfaces. In: Biochemistry and molecular biology of parasites, 1sted. *Academic press, New York*. 1995; 203-232.

26. 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 freeliving nematode *Caenorhabditis elegans*. *Parasites & Vectors*. 2012; 190:136-142.

27. Koga MD, Nveikoueing F, Bambe R, Mando NB, Nukenine EN, Ndjonka D. Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon). *Cameroon Journal of Biological and Biochemical Sciences*. 2020; 28(2):14-25.

28. Ndjonka D, Abladam ED, Djafsia B, Ajonina-Ekoti I, Achukwi MD, Liebau E. Anthelmintic activity of phenolic acids from the axlewood tree *Anogeissus leiocarpus* on the filarial nematode *Onchocerca ochengi* and drug-resistant strains of the free-living nematode

Caenorhabditis elegans. *Journal of Helminthology*. 2013; 1-8. doi:10.1017/S0022149X1300045X.

29. Katiki LM, Ferreira JFS, Gonzalez JM, Zajac AM, Lindsay DS, Chagas ACS, Amarante AFT. Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. *Vet. Parasitol*. 2012; 192(1-3):218-227. http://dx.doi.org/10.1016/j.vetpar.2012.09.030.

30. Akongwi M, Tih E, Nyongbela KD, Samje M, Ghogomu RT, Bernard B. Brevipedicelones D and E, Two C–O–C Flavonoid Dimmers from the Leaves of *Garcinia brevipedicellata* and Anti-onchocercal Activity. *Natural Products and Bioprospecting*. 2018; 9:61-68. https://doi.org/10.1007/s13659-018-0191-9.

31. Moshi MJ. Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *Afr. J. Tradit. Complement. Altern. Med*. 2007; 4:219-225.

32. Bulgheroni A, Kinsner-Ovaskainen A, Hofmann S, Hartung T, Prieto P. Estimation of acute oral toxicity using the No observed adverse efect level (NOAEL) from the 28-day repeated dose toxicity studies in rats. *Regul Toxicol Pharmacol*. 2009; 53(1):16-19

33. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology*. 2000; 32:56-67.

34. Ramaiah SK. Preclinical safety assessment: current gaps, challenges, and approaches in identifying translatable biomarkers of drug-induced liver injury. *Clinics in Laboratory Medicine*. 2011; 31(1):161-172.

35. Manda P, Manda O, Vangah-Manda MO, Kroa E, Dano SD. Étude des toxicités aigue et subaiguë du remède NATURE utilise dans le traitement du paludisme. Rev. Ivoir. Sci. Technol. 2017; 29:145-158. ISSN 1813-3290. http://www.revist.c.

36. Mando NB, Bambe R, Djongra M, Koga M-D, Ndjonka D. Phytochemical Analysis and Anthelmintic Activity of Ethanolic Extracts of *Gmelina arborea* on the Bovine Parasite *Onchocerca ochengi*. *Journal of Pharmaceutical Research International*. 2022; 34(46): 45-61. Article no.JPRI.89412. ISSN: 2456-9119.

37. Tédong L, Dzeufiet DDP, Dimo T, Asongalem AE, Sokeng NS, Flejou J-F, Callard P, Kamtchouing P. Acute and subchronic toxicity of *Anacardium occidentale* Linn (anacardiaceae) leaves hexane extract in mice. *fr. J. Trad. CAM*. 2007; 4(2):140-147.

38. Betti AH, Stein CA, Dallegrave E, Barth WTA, Negrao WTT, Driemeier D, Buffon A, Kuze RSM. Acute and repeated-doses (28 days) toxicity study of *Hypericum Polyanthemum* Klotzsch ex Reichardt (Guttiferare) in mice. *Food and Chemical Toxicology*. 2012; 2012:2349-2355.

39. Ouahchia C, Cherif H-S, Hamaidi-CF, Marzen L, Deradji S, Hemma R, Nouar N, Saidi F. Toxicité aiguë et subaiguë des extraits méthanoliques d'Inula viscosa L. (dittrichia viscosa L.). *Revue Agrobiologia*. 2017; 7(2):562-573.

40. Ezeonwumelu JOC, Julius AK, Muhoho CN, Ajayi AM, Oyewale AA, Tanayen JK, Balogun SO, Ibrahim A, Adzu A, Adiukwu CP, Oloro J, Kiplagat DM, Goji ADT, Okoruwa AG, Onchweri AN, Reddy PMK. Biochemical and histological studies of aqueous extract of Bidens pilosa leaves from Ugandan Rift Valley in Rats. British Journal of *Pharmacology and Toxicology*. 2011; 2(6):302-309.

41. Azza DHD, Mohamed EHS, Sara MEHS. Acute Toxicity Studies of Ethanolic Extract of Eucalyptus Camaldulensis Dehnh Leaves Acute Toxicity Studies of Ethanolic Extract of *Eucalyptus Camaldulensis* Dehnh Leaves. 2015.

42. Reichl FX. Guide pratique de toxicologie. 2nd ed. DeBoeck, Larcier (Bruxelles). 2004; 4-16

43. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, Umar M, Njan AA. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. Heliyon. 2019; 5:1-20. doi: 10.1016/j.heliyon.2019. e01179.

44. Kabiru YA, Okogun JI, Gbodi TA, Makun HA, Ogbadoyi EO. Subchronic Toxicity Studies of Methanol Extract of *Eucalyptus Camaldulensis* (Leaf) In Wister Rats. Iosr Journal of Pharmacy. 2013; 3(2):38-45.

45. Taylor JLS, Rabe T, McGraw LJ, Jäger AK, Staden J. Towards the scientific validation of traditional medicinal plants. *J. Plant growth Regulation*. 2001; 34:23-37.

46. Osei-Bimpong A, McLean R, Bhonda E, Lewis SM. The use of the white cell count and haemoglobin in combination as an effective screen to predict the normality of the full blood count. *Int. J. Lab. Hematol*. 2012; 34(1):91-97.

47. Ntchapda F, Abakar D, Kom B, Nana P, Hamadjida A, Dimo T. Acute and sub-chronic oral toxicity - assessment of the aqueous extract leaves of *Ficus glumosa* Del. (Moraceae) in rodents. *Journal of Intercultural Ethnopharmacology*. 2014; 3(4):20621.