

Research Article



Cameroon Journal of Biological and Biochemical Sciences 2020, Vol 28, Serie 2, 14-25

ISSN 1011-6451/CJBBS.2019. Published Online (February 2019) (www.camjournal-s.com)

Email : koga2006@yahoo.fr, ndjonka_dee@yahoo.com, Tel : 237697759347, 237691677064

Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

KOGA Mang'Dobara^{1,2*}, NVEIKOUEING Francis¹, BAMBE Raymond¹, MANDO Ndou Bertrand¹, NUKENINE Elias Nchiwan¹, NDJONKA Dieudonne^{1*}.

1: University of Ngaoundere; 2: University of Yaounde I; *: Corresponding author.

(Submitted on the 1st of September 2019, accepted on the 31st of January 2020)

Résumé

Environ 18 millions de personnes sont infectées par *Onchocerca volvulus* à travers le monde. L'ivermectine (IVM), médicament de choix utilisé dans le traitement de l'onchocercose est essentiellement microfilaricide et induit des effets indésirables importants notamment chez les malades présentant une charge microfilarienne élevée et dans les zones où l'onchocercose et la loase sont co-endémiques. Dans le but de contribuer au développement de nouvelles molécules anti-onchocercariennes moins toxiques et actives sur les formes adultes du parasite *O. volvulus*, l'extrait méthanolique de *Canarium schweinfurthii* a été testé *in vitro* sur le parasite des bovins *Onchocerca ochengi*. Les vers mâles adultes d'*O. ochengi* ont été incubés à 37° C dans le milieu de culture Roosevelt Parck Memorial Institut (RPMI)-1640 enrichi à la Pénicilline et Streptomycine, en présence de l'extrait de la plante à des différentes concentrations. L'IVM et le RPMI ont servi respectivement des témoins positif et négatif. La mortalité des vers a été déterminée après 48h et 72h d'incubation par le test colorimétrique au Methyl Tetrazolium (MTT). La toxicité aigüe de la plante a été évaluée en administrant aux rats albinos de souche Wistar une dose unique de 2000 mg/kg de l'extrait puis, les paramètres biochimiques des animaux ont été dosés après 14 jours d'observation. L'extrait méthanolique de *C. schweinfurthii* ainsi que l'IVM ont été létaux pour les parasites. Les concentrations nécessaires pour induire 50% de mortalité (CL₅₀) des vers incubés à 72 heures étaient de 146 ± 34,6 µg/ml pour l'extrait de la plante et de 55.5 ± 8.1 µg/ml pour le control positif. L'analyse phytochimique de l'extrait a révélé la présence des alcaloïdes, composés phénoliques, saponines, quinones, terpénoïdes et glycosides. Aucune mortalité n'a été enregistrée à l'issue de l'étude de la toxicité aigüe et la valeur dose de l'extrait nécessaire pour induire 50% de mortalité (DL₅₀) des rats traités était supérieure à 2000 mg/kg. La comparaison des valeurs des paramètres biochimiques des rats traités et ceux du lot témoin négatif n'ont montré aucune différence significative (P > 0,05).

Ces résultats attestent que l'extrait méthanolique de *C. schweinfurthii* peut servir de source pour le développement de nouvelles molécules anti onchocercariennes et justifient l'utilisation de la plante dans le traitement des maladies parasitaires et plusieurs autres affections par les populations locales.

Mots clés : *Onchocerca volvulus* ; *Canarium schweinfurthii* ; LC₅₀ ; Toxicité aigüe ; Paramètres biochimiques.

Abstract

An estimated 18 million people carry dermal *Onchocerca volvulus* around the world. Ivermectin (IVM), the current drug of choice for the treatment of the disease is only microfilaricidal and several adverse effects of this molecule have been reported notably in people with high microfilariae densities and in areas where onchocerciasis and loaisis are co-endemic. Thus, in contributing to the development of new products, safe and

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

active against *O. volvulus* adult worms, *Canarium schweinfurthii* methanolic extract was screened *in vitro* on the adult bovine parasite *Onchocerca onchengi*. Extracted worms were incubated at 37 °C, in RPMI-1640 supplemented with Penicillin and Streptomycin in the presence of plant extracts at different concentrations. Ivermectin and RPMI served as positive and negative controls respectively. Worm's mortality was assessed biochemically after 48 h and 72 h using the Dimethylthiazol (MTT)/Formazan colorimetric assay. Acute oral toxicity of plant extracts was evaluated in albino wistar rats with a single dose 2000 mg/kg body weight then biochemical parameters were assessed after 14 days of observation period. *C. schweinfurthii* extract and also ivermectin exhibited anti-onchocercal activities. Concentrations required to kill 50 % of incubated worms (LC₅₀) after 72 hours were 146.0 ± 34.6 µg/ml and 55.5 ± 8.1 µg/ml for the plant extract and positive control respectively. Phytochemical analysis of the extract revealed the presence of alkaloids, phenolic compounds, flavonoids, saponins, quinones, terpenoids and glycosides. The results of the acute toxicity showed no mortality and LD₅₀ cut off value was observed to be >2000 mg/kg bw and there was no significant difference (P > 0.05) between the treated groups and control group in all assessed parameters.

These findings suggest that methanolic extract of *Canarium schweinfurthii* can serve as source of new anti-onchocercal product development and validate the use of the plant by local populations for the treatment of parasitic disease and many other ailments.

Key words: *Onchocerca volvulus*; *Canarium schweinfurthii*; LC₅₀; Acute oral toxicity; biochemical parameters.

INTRODUCTION

Human onchocerciasis, commonly referred to as River Blindness in Africa and Robbles Disease in the Americas is caused by the filarial nematode, *Onchocerca volvulus* (Leuckart) Railliet & Henry, 1910 (Cupp et al., 2011). This Parasite, first described by Leuckart in 1893, is transmitted by Simulium sp black flies, intermediate hosts that require fast-flowing water for their breeding and development (Bazanez et al., 2006). The large adult female worms are contained within fibrous nodules or onchocermas in subcutaneous or deeper tissues of infected people. Males migrate between nodules to inseminate the females, which when fertilized give birth for 12 to 14 years, to millions of microfilariae, which constitute the parasitic pathogenic stage (Taylor et al., 2010). The disease is associated with significant and unsightly skin changes, impaired vision that can lead to blindness, musculoskeletal pains, reduced body mass index, infantile dwarfism known as Nakalanga syndrome (Noma et al., 2002; Adrian and Boakye, 2011). About 18 million people carry dermal *O. volvulus* microfilariae worldwide with 99 % of them living in Africa (Yaya et al., 2014). The mainstay of onchocerciasis control is through antivectorial and antiparasitic measures. The former is directed against the black fly aquatic stages, and the latter against the microfilariae (Bazanez et al., 2006). At present, only Ivermectin (IVM) (Mectizan) is recommended for chemotherapy and for mass drug administration (Samje et al., 2014). This molecule temporally sterilizes adult female worms and is active on dermal stages only. Several adverse effects of ivermectin have been reported. Skin reactions after receipt of ivermectin treatment are commonly observed in persons with high microfilariae densities (Cooper et al., 1999); in those area where onchocerciasis and loiasis (caused by filarial nematode *Loa loa*) are coendemic

(mainly in central Afrique), ivermectin treatment for *O. volvulus* in individuals with high *L. loa* microfilariae leads to several adverse events including fatal encephalopathy (Gardon et al., 1997); exposure of *O. volvulus* adult worm to ivermectin caused genetic selection associated with a lower reproductive rate in the female parasites which could have implications for the development of ivermectin resistance (Bourguinat et al., 2007). Thus, the search of new molecules, safe and active against adult worms of onchocerciasis adult worms is necessary. Medicinal plants, generally used in traditional medicine for the treatment of many ailments could therefore constitute one of the interesting lines for the development of new filaricidal compounds (Fabricant et Farnsworth, 2001; Ogukwe et al. 2004; Ndjonga et al., 2013). The nematocidal effects of several plant species have been documented (Wabo et al; 2005; Ndjonga et al., 2011). The Plant used in this study has been selected after an ethnobotanical survey conducted at Mvog Atangana Mballa market in Yaounde (Center Region of Cameroon). *C. schweinfurthii* has been shown to express antioxidant (Ayoade et al., 2015), antibacterial (Gangoue et al., 2009; Damien et al., 2016) and hypoglycemic (Kamtchouing et al., 2008) properties. The 3 β-Hydroxylolean-12,18-diene isolated from the aerial parts of the plant has been shown to inhibit the developmental stages of *Ascaris suum* (Okoli et al., 2016). In our knowledge, the anti onchocercal property of the above plant has not yet been documented.

We herein report the anti-onchocercal activity of *C. schweinfurthii* against *O. ochengi* male adult worms. Additionally, we report on its *in vivo* toxicity.

MATERIAL AND METHODS

Plant material and chemicals

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

If not stated otherwise, all chemicals were purchased from Sigma (Deisenhofen, Germany). Plant material consisted of *Canarium shweinfurthii* root bark, collected early in the morning from Mfou in the Center Region of Cameroon, in the month of June 2017.

Animal materials

Animal materials consisted of female Wistar nulliparous and non-pregnant rats weighing between 88 and 150g, aged between 08 to 12 weeks obtained from the National Veterinary Laboratory (LANAVET, Garoua, Cameroon) and fresh pieces of umbilical cattle skin containing large number of palpable nodules obtained from Ngaoundere Municipal slaughterhouse.

METHODS

Collection and preparation of plant materials

Canarium schweinfurthii root barks were collected from Mfou (11°64'26.86'' N and 3°71'57.51'' E), Center Region of Cameroon, in June 2017. A voucher specimen of the plant was deposited at the National Herbarium, Yaounde, Cameroon, identified by Mr Eric Ngansop in comparison with R8763 Letouzey voucher; Benth No 16929/SRF/Cam. Plant material was then carefully washed with tap water and dried under shade to avoid destruction of active compounds (Ezekwe *et al.*, 2013). The dried material was pounded in a mortar, sieved and the resulting powder was stored until the extract preparation.

Preparation of methanolic extract

Plant's extract preparation was carried out by the method described by Aswanida (2015) with slight modifications. 100 g of the powder was macerated in 70 % methanol -30 % water (v/v) for 48 h. The mixture was centrifuged (3,500 x g, 10 minutes), filtered over filter papers and concentrated in a rotary evaporator (BUCHI Rotavapor R-200, Zwitzerland) at 35 ° C, under reduced pressure. The concentrated was dried at 40 °C in a vacuum and the resulting solid extract was stored in a refrigerator at 4 °C for further investigation.

Preparation of plant extracts and reference stock solutions

To prepare plant extract stock solution, 0.1 g of the dry extract was introduced into sterile falcon tube. Thereafter, 100 mL of distilled water were added. For the reference, solution of 10 mg/mL Ivermectin (IVM) was diluted with distilled water to a final concentration of 1 mg/mL. The

mixtures were homogenized (Vortex, Heidolph) and then stored at 4°C for further investigation.

Isolation of *Onchocerca ochengi* adult worms

The isolation of adult worms of *O. ochengi* was carried out according to the protocol described by Cho-Ngwa *et al.* (2010) with slight modifications. Fresh pieces from umbilical cattle skin with palpable *O. ochengi* nodules, collected from the Ngaoundere Municipal slaughter house were directly transported to the Laboratory of the University of Ngaoundere (Cameroon) and then washed with tap water and sterilized with 70 % ethanol which was allowed to evaporate. Individual nodules were isolated from the skin using a scalpel blade and washed three times with sterile phosphate-buffered saline (PBS-pH 7.4). *O. ochengi* adult worm masses containing adult worms were recovered by careful excision of the nodules under a dissecting microscope, using raised needle and forceps. The extracted male worms were washed twice in Phosphate Buffered Saline (PBS) and RPMI (Roosevelt Parck Memorial Institut) 1640 culture medium supplemented with 25 µg/mL penicillin and streptomycin.

Anti-onchocercal screening of plant extract

This was conducted following the protocol of Borakaeyabe *et al.* (2015). Stock solution of plant extract was dissolved into RPMI 1640 to get different tested concentrations (0; 0.1; 0.25; 0.375; 0.5; 0.625 and 1mg/mL). IVM (60, 80, 100, 120, 150 and 170 µg/mL in RPMI 1640) and RPMI-1640 culture medium were used as positive and negative controls respectively. 100 µL of each mixture (RPMI 1640 + plant extract or RPMI 1640 + IVM) and RPMI 1640 were transferred into a culture well before adding worm. Adult male worms (one individual per well) were incubated with different concentrations of plant extracts (0; 0.1; 0.25; 0.375; 0.5; 0.625 and 1mg/ml), IVM (60, 80, 100, 120, 150 and 170 µg/mL) and RMPI (without plant extract and IVM) in 96 -well plates. Each test consisted of six worms in six different wells. Assays were incubated at 37 °C and mortality was recorded after 48h and 72 h (Ndjonka *et al.*, 2012). Trials were conducted in three independent tests.

Assessment of adult worms' viability

Adult male worms' viability was assessed by the standard colorimetric Dimethylthiazol (MTT)/Formazan assay (Samje *et al.*, 2014). MTT is a pale yellow compound which is reduced to a dark blue product, formazan by mitochondrial enzymes of living cells (Gallambos *et al.*, 2015). After incubation, worms were removed from the 96-wells plate, washed in PBS and placed in a 24-well plate (six individuals for the same test per well) containing 500 µL of 0.5 mg/mL of MTT in RPMI 1640 per well, followed by incubation at 37

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

°C in the dark for 30 minutes (Borakaeyabe *et al.*, 2015) and then observed under a dissecting microscope. The concentration required to kill 50 % of incubated worms (LC₅₀), for the plant part extract and the reference was determined after recording parasite mortality.

Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out on 0.1 mg/mL of plant extract solution. The extract was assessed for the presence of phenolic compounds, flavonoids, alkaloids, quinones, glycosides and saponins. Detection of these secondary metabolites was performed according to N'Guessan *et al.* (2009). Briefly, phenolic compounds were detected by adding three drops of 10 % ferric chloride to the extract, formation of a greenish colouration was a positive reaction. Flavonoids were determined using magnesium chips and 1 mL of HCl, an orange, red or purple colour was taken as a positive test. For the presence of alkaloids, 1 mL of 5% HCl and 3 drops of Dragendorff reagent were added to 1 mL of the extract and the mixture observed for white or orange precipitate. The presence of quinones was determined using 2 mL of petroleum/ether mixture and 2 mL of 10% NaOH, red colouration indicated a positive control. Glycosides were revealed by adding to 1 mL of the extract solution, 1 mL of chloroform, 1 mL of acetic acid. A purple, blue or green ring indicated a positive reaction. For saponins assessment, 5 mL of the extract was shaken vigorously with 5mL of distilled water in test tube and then allowed to stand for 15 minutes. Formation of stable foam, height greater than 1 cm was taken as an indication for the presence of these compounds.

Quantitative phytochemical analysis

Total phenolic content

Total phenol content was determined by using the Folin and Ciocalteu (FC) method. The various concentrations (0.02- 0.15 mg/mL) of standard (gallic acid) and plant extract were prepared with the reaction mixture containing 100 µL of gallic acid, 500 µL of F C reagent and 400 µL of 7.5% sodium carbonate. The mixture was then incubated at room temperature for 10 minutes and the absorbance was read using a spectrophotometer (spectrophotometer UV, Dna) at 730 nm (Nathalie and Jean-Paul, 2006). The total content of phenol compounds in gallic acid equivalent was determined from the calibration curve of gallic acid and expressed in mg gallic acid equivalent (GAE)/g of plant extract.

Total flavonoid content

The estimation of total flavonoid content was carried out by the method of Dehpour *et al.* (2006). Different concentrations (0.02- 0.15 mg/mL) of standard (rutin) and plant extract were

prepared with 500µL rutin added to 1500 µl of 95% (v/v) methanol, 100 µL of 10 % (m/v) Aluminium Chloride (AlCl₃), 100 µL of 1M sodium acetate (CH₃COONa), 2.8 mL of distilled water. The mixture was stirred and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 415 nm, using a spectrophotometer (spectrophotometer UV, Dna). Results were determined from the calibration curve equation of rutin and expressed in mg rutin equivalent (RE)/g of dry extract.

Condensed tannins

Condensed tannins content was determined by the vanillin hydrochloride method of Ba *et al.* (2010). The reagent was prepared by mixing equal volume of 8 % (v/v) hydrochloric acid (HCl), 37% (v/v) methanol and 4% vanillin in methanol (m/v). The mixture was kept at 30°C before the assay. 200 µL of plant extract or catechin at different concentrations (0.06 -0.3 mg/mL) were added to 1000 µL of vanillin reagent mixture and incubated in the dark at 30°C for 20 minutes. The absorbance was measured at 500 nm using a spectrophotometer. Total contents of condensed tannins in catechin was calculated from catechin calibration curve and expressed in mg catechin equivalent (Cat E) /g of plant extract).

Breeding and maintenance of rats

The rats were acclimatized for one week under standard environmental condition, with 12 hours light/dark cycle maintained on a regular vital feed and water.

Acute Oral toxicity studies

Acute oral toxicity of *C. schweinfurthii* root bark extract was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline 423 for testing of chemicals following their application as assays, limit the single dose of 2000 mg/kg body weight. Extract was dissolved in distilled water to a final concentration of 200 mg/mL corresponding to 2000 mg/kg body weight dose. Prior to the administration of plant extract, animals were withheld from food overnight but not from water. The animals were weighed and divided into two groups (01 treated group and 01 control group) of three individuals each. Then, 2000 mg/kg bw dose of the extract was orally given with to the treated groups in a single dose/exposure, while the untreated group received only distilled water. After plant extract administration, Wistar albino rats were withheld from food for another 03 hours and observed closely for changes in skin, somatomotor activity, diarrhea, sleep and mortality for the first four hours, then over a period of 24 hours and thereafter twice daily for 14 days. Weight changes were also

recorded weekly during the observation period. At the end of the acute toxicity studies, animals were anesthetized with ether and then sacrificed. Blood samples were collected for biochemical parameters assessment.

Assessment of biochemical parameters

The serum biochemical parameters including albumin, creatinine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were evaluated spectrophotometrically using an automatic analyzer.

Statistical analysis

Results were expressed as the mean value \pm standard error of the mean (SEM) and were subjected to one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test.

RESULTS

Activity of plant extracts on *Onchocerca ochengi* adult worms

Mortality rates of *O. ochengi* 48 h and 72 h after incubation are presented in figure 1. Results revealed that both *C. schweinfurthii* root bark extract (Figure 1A) and the reference (Figure 1B) induced *O. ochengi* mortality in a time and concentration-dependent manner. No mortality was recorded in negative control. At the lowest concentration (ie 100 μ g/mL), the plant extract induced 22.21% and 44.44% of parasite mortality after 48 h and 72 h respectively. Whatever the concentration and the incubation time, Ivermectin induced higher mortality worms compared to the plant extract. Lethal concentrations required to kill 50% of incubated worms (LC_{50}), calculated from mortality rates are presented in figure 2. After 48h and 72h the LC_{50} were $306.3 \pm 62.1 \mu$ g/ml and $146.0 \pm 39.0 \mu$ g/ml respectively. Positive control was most active against *O. ochengi* with LC_{50} values $76.2 \pm 5.0 \mu$ g/mL and $55.5 \pm 8.1 \mu$ g/mL after 48 h and 72h.

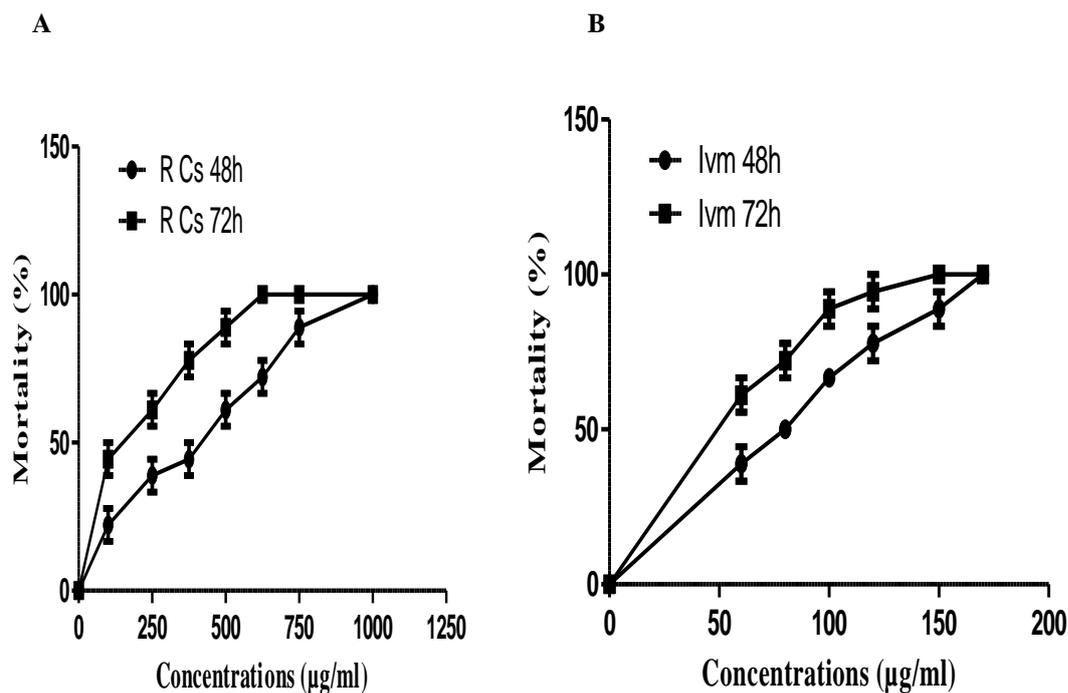


Figure 1: Mortality rates of *O.ochengi* after exposure to *C. schweinfurthii* root bark extract and Ivermectin.

R Cs: root bark of *C.schweinfurthii* ; Ivm : Ivermectin. Data are mean \pm standard error of the mean (SEM)

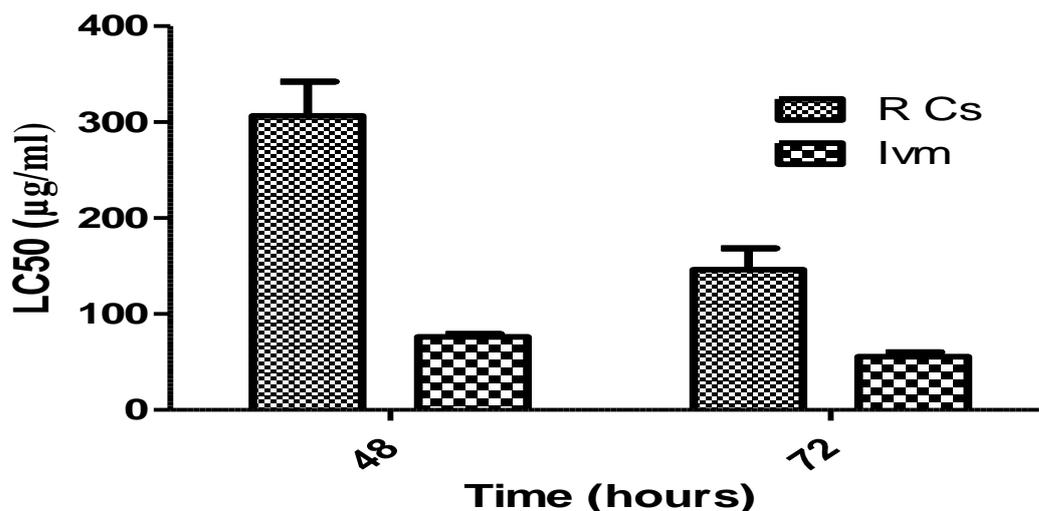


Figure 2: Lethal concentrations values (LC₅₀) values of plant extracts and Ivermectin.

Phytochemical screening

Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins, anthraquinones, terpenoids and glycoside. For the quantitative phytochemical screening, flavonoids, Phenolic compounds and condensed tannins contents were determined from their respective equation of calibration curves. Results shown that the total phenolic compounds were more abundant (130.97 ± 0.25 mg GAE/ g of plant extract) followed by condensed tannins (10.83 ± 0.21 mg Cat E/g plant extract). Flavonoids contents were the less

abundant metabolites (5.17 ± 0.11 mg RE/g plant extract).

Effect of plant extracts on animals biochemical parameters

Results of the effect of single dose of 2000 mg/kg bw of the plant extract on biochemical parameters are presented in table 1.

The results indicated a significant decreased ($P < 0.05$) in creatinine value compared to the negative control. For the other parameters (ALT, AST, Albumin) values, no significant difference was observed ($P > 0.05$) between the two groups.

Table 1: Biochemical parameters of control and treated animal groups

Parameters	Treatment group	
	Control group	RCs
Albumin (g/L)	34.6 ± 0.3^a	37.0 ± 1.2^{ab}
Creatinine (mg/L)	0.4 ± 0.1^b	0.2 ± 0.1^a
AST (UI/L)	19.0 ± 2.0^a	18.3 ± 2.1^a
ALT (UI/L)	51.7 ± 2.1^b	49.3 ± 3.8^{ab}

AST: Aspartate aminotransferase; **ALAT:** Alanine aminotransferase. Values are means \pm SEM. Means in the same line followed by the same letter are not significantly different ($P > 0.05$).

DISCUSSION

The aim of the present study was to assess filaricidal activities of *C. schweinfurthii* extract on *O. ochengi*. In our opinion, this paper reports for the first time the anti-onchocercal effect of the plant.

Results of our study revealed that the mortality rates recorded at 48 h and 72 h after incubation were dose dependent. Plant extracts and also IVM showed filaricidal activities against *O. ochengi*. Several molecules belonging to the detected molecule classes were found active on nematodes. The presence of these secondary metabolites such as flavonoids, tannins, saponins, quinones in the studied plant extract can provide a preliminary explanation on its filaricidal activities. Synthetic anthelmintic target parasites cell membrane and exert their effect in various way, resulting in the paralysis followed by the death of the worm. As examples, IVM affects nematode motility, feeding, and reproduction and acts via ligand-gated chloride channels, specifically those gated by glutamate (Yates, 2003); Diethylcarbamazine immobilizes worms due to a possible hyperpolarization followed by a change on the surface of parasites, rendering them susceptible to the immune host (Florencio *et al.*, 2003); Albendazole causes degenerative alterations in the intestine cells of the worm by binding the colchicine-sensitive site of β -tubulin, thus inhibiting its polymerization, leading to impaired uptake of glucose by larval and adult stages of the parasite (Verrest and Dorlo, 2017); Suramin exhibits its action through a range of enzymatic reactions, disrupting cell growth via interference with DNA-RNA replication (Andy *et al.*, 2012). Plants bioactive compounds would exert their nematocidal activities by one or other of the evoked mechanisms. The possible effects of condensed tannins on nematodes are related to their ability to form complexes and create interactions with parasite surface proteins, disrupting essential function such as nutrition and reproduction (Molan *et al.*, 2004; Rahman et Seip, 2007). Saponins are known to interact with the cell membranes causing changes in cell wall permeability (Ademola and Ellof, 2010; Doligasika *et al.*, 2011), they also interact with the collagen cuticle of nematodes and this interaction may be responsible for the nematotoxic effect observed in our data (Argentieri *et al.*, 2008); alkaloids exert their activity by intercalating into the cell wall and DNA parasites (Patel *et al.*, 2010). *O. ochengi*, as most other filarial nematodes harbor an intracellular bacterial symbiont (Wolbachia), it is thus susceptible to some antibiotic classes' action (Illona *et al.*, 2006; Francesca *et al.*, 2011). Antibiotic effects of the plant used in this study have been elucidated. *C. schweinfurthii* stem bark ethanolic extract was shown to inhibit both positive and negative gram

bacteria growth (Damien *et al.*, 2016); in a study conducted by Sokoudjou *et al.* (2018), decoction, infusion and ethanolic extract of *C. schweinfurthii* extract, were found to be active against some *Salmonella* strains. Active compounds found in our plant extract such as tannins, saponins, flavonoids, alkaloids, quinones may also exert this antibacterial activity (Jacob *et al.*, 1991; Hasanori *et al.*, 2001; Cushnie and Lamb, 2005; Strauss and Hancock, 2006; Omajate *et al.*, 2014).

Phytochemical screening revealed the presence of alkaloids, phenolic compounds, flavonoids, terpenoids, glycosides saponins and quinones in the plant extract. This observation is in concordance with the results of Ayoade *et al.* (2015) who reported the presence of the same secondary metabolites in *C. schweinfurthii* fruits ethanolic extract. A recent study conducted by Adesina and Rajashekar (2018) confirmed the presence flavonoids, saponins and glycosides in aqueous leaves extract of *C. schweinfurthii*. However, our results were in contrast with those of Okoli *et al.* (2015) who reported the absence of alkaloids in chloroform leaves extract of *C. schweinfurthii*. These differences in phytochemical composition of the above two plant could be attributed to differences concerning the plant development stage at the harvest period, conditions related to the harvesting area, the harvest season, the extraction method, the nature of the solvent used (Ncube *et al.*, 2008; Dedehou *et al.*, 2014; Osemwegie and Dahun., 2015). Secondary metabolites contents of various *C. schweinfurthii* extracts had been estimated by Sokoudjou *et al.* (2018) in plants collected from Bamougoum, West region of Cameroon. They obtained higher quantities of phenolic compounds and condensed tannins in ethanolic (416.29 ± 10.09 mg GAE/g of dry extract for total phenolic compounds and 37.97 ± 0.90 mg CatE/g of dry extract for the condensed tannins) and hydroethanolic (410.68 ± 23.56 mg EAG/g of dry extract for total phenol compounds and 48.37 ± 1.57 mg CatE/g of dry extract for the condensed tannins) stem bark extracts compared to that in our study. Other studies obtained lower secondary metabolites quantities compared to ours. In a study conducted by Damien *et al.* (2016), secondary metabolites quantities in *C. schweinfurthii* stem bark ethanolic extract were: 92.22 ± 0.19 μ g GAE/g; 2.36 ± 0.12 μ g QE/g; 0.95 ± 0.03 μ g TAE/g for phenolic compounds, flavonoids and tannins contents respectively. The observed differences in secondary metabolites quantities in our study and those of the above authors could be due to environmental factors such as light, temperature, water soil fertility and salinity (Nenad *et al.*, 2017). Thus, it had been confirmed that quantity of polyphenols contents in certain plants increases due to the stress caused by greater concentrations of salt in ground substrate (Queslati

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

et al., 2010). The quantitative distribution of secondary metabolites in extracts can also be influenced by the extraction temperature, the solvent polarity which allows the solubilisation of compounds of similar polarity and the solvent concentration (Prastan *et al.*, 2011).

Results from toxicological study of our plant extract showed that no mortality was recorded for the 14 days observation period, indicating that the DL₅₀ value of the tested extract was up to 2000 mg/kg bw, and this puts the extracts in category 5 toxicity class according to the Globally Harmonised System (GHS) of classifying substances (OCDE 423) which are regarded as relatively safe substances. Our results are in line with those of Nnama *et al.* (2017) who observed no mortality after oral administration of a single dose of 5000 mg/kg bw of ethanolic leaves extract of *Erythrina senegalensis* to albino Wistar rats. In another study conducted by Ndjonka *et al.* (2017), 33.3% of mortality was recorded following oral administration of 1500 mg/kg bw dose of *Trichelia emetica* ethanolic extract to BALB/c mice. Extract chemical composition could explain the toxicity signs and mortality recorded in the evaluation of Ndjonka *et al.* (2017).

Assessment of Albumin is an important criterion to examine the synthetic and excretory function of the liver (2000; Yakubu *et al.*, 2003). It has been reported that low proteins level results when there is extensive liver damage (Wada and Snell., 1962). In this study, the increase in albumin value observed in the treated group proves that, the single dose of 2000 mg/kg bw of *C. schweinfurthii* extract did not impair liver function of experimental animals. Onu *et al.* (2013) found different results from ours. They reported that oral administration of 2000 mg/kg bw of aqueous extract of the stem bark of *Khaya senegalensis* induced significant decrease of albumin values in treated rats, compared to the control group.

Aminotransferases (ALT and AST) are hepatic dysfunction markers and are thus used for the diagnostic of liver cytolysis (Hassan *et al.*, 2008). The transaminases values in the treated group were not statistically different ($P > 0.05$) compared to the control group. This attested that the 2000 mg/kg bw dose of our plant extract did not produce adverse effect on the liver function. This is in accordance with the non-significant difference ($P > 0.05$) in the same parameters observed by Coulibaly *et al.* (2018) after oral administration of 2000 mg/kg bw dose of *Parkia biglobosa* ethanolic seed extract to albino rats. Onu *et al.* (2013) reported significant increase ($P < 0.05$) of transaminases values in treated animal groups when compared to the untreated group after oral administration of 2000 mg/kg bw of aqueous extract of the stem bark of *Khaya senegalensis*.

According to Narayana *et al.* (2001), flavonoids have been found to possess hepato-protective activity. The results of several clinical investigations showed the efficacy and safety of flavonoids in the treatment of hepato-biliary dysfunction and digestive disorder (Ruiyc, 1991).

Creatinine is a non-protein nitrogenous metabolite that is cleared from the body by the kidney, following glomerular filtration (Nwankpa *et al.*, 2018). The estimation of the level of this metabolite is employed as marker for kidney function (Yakubu *et al.*, 2003). Assessment of creatinine level showed that there was a significant decrease ($P < 0.05$) in the animals treated group compared to the control group. Decrease in creatinine values observed in the treated group suggested that glomerular function of the treated animals was not affected. Okwuoso *et al.* (2009) demonstrated that aqueous and methanolic extracts of *C. schweinfurthii* have a role in protecting rats from Acetaminophen induced renal injuries. This may justify the significant reduction of creatinine level observed in the treated group. Lydia *et al.* (2017) also noted a significant decrease ($P < 0.05$) of creatinine values in rats orally treated with 1000 mg/kg bw dose of *Launea taraxacifolia* ethanolic leaves extract compared to the control group. Significant increase ($P < 0.05$) of creatinine values in male rats treated with 300 mg/kg bw *Datura metel* L ethanolic extract compared to their control group have been reported in a study conducted by Chinedu *et al.* (2019). High levels of creatinine are found in renal injury, which could be attributed to some groups of saponin or alkaloid (Iheme *et al.*, 2013).

CONCLUSION

This study reveals that *C. schweinfurthii* exhibits filaricidal activities on *O. ochengi* adult worms. Its efficiency could be attributed to the compounds such as condensed tannins, saponins, quinones, flavonoids and alkaloids. Toxicity profiles shows no mortality and harmful effect in rats using single dose of 2000 mg/kg bw. These findings suggest that the studied plant can serve as a source of new anti-onchocercal development product and validate its use in traditional medicine for the treatment of parasitic diseases and several other ailments.

CONSENT

It is not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

ACKNOWLEDGEMENTS

The authors appreciate herb sellers of the Mvog Atangana Mballa Market of Yaounde (Cameroon). They are grateful to the inhabitants of Mfou (Yaounde). They would like to thank M. ERIC Nganso of the National Herbarium of Cameroon who helps them to identify the plant. They are also very grateful to the Alexander von Humboldt Foundation (AvH) for their material support.

REFERENCES

Ademola O.I., Elff J.N., 2010. *In vitro* anthelmintic activity of activity of *Combretum molle* (R. Br. ExG. Don) (Combretaceae) against *Haemonchus contortus* Ova and Larvae. *VeterinaryParasitology*. 169: 198-203.

Adesina J.M., Rajashekar., 2018. Phytochemical composition and insecticidal potentials of some plant aqueous extracts in suppressing *Podagrica spp* (Coleoptera:Chrysomelidae) infestation on Okra (*Abelmoschus esculentus* Moench). *Advances in orticultural Science*. 32 (1):71-78.

Adrian Hopkins., Bakye A. Boatin. 2011. Water and Sanitation-Related Diseases and the Environment: Challenges, Interventions, and Preventive Measures. First edition. 133-150.

Andy Crump, Carlos M.M., Satoshi Omura, 2012. The onchocerciasis Chronicle: from the beginnig to the end? *Trends in parasitology*; 28 (7): 279-288.

Argentieri P.M., D'Addabe T., Tava A., Agostinelli A., Jurzysta M., Anato P., 2008. Evaluation of nematicidal properties of saponins from *Medicagosativa spp*. *European Journalof Plant Pathology*. 120: 189-197.

Aswanida N.N, 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal andAromatic plants*: 4 (3): 1-6.

Ayoade G.W., Amoo I.A., Gbolahan-Ayoade E.E., 2015. Phytochemical composition and antioxidative potential of purple Canary (*Canarium schweinfurthii*) fruit. *The Pharma Innovation Journal*. 4(1) : 49-52.

Ba K., Tine E., Destain J., Cisse N., Thonart P., 2010. Etude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt. *Biotechnology, Agronomy, Society and Environnment*.14: 131-139.

Bazanez M.G., Sébastien D.S.P., Thomas S.C., Lutz P.B., Mark P.L., Michel B., 2006. River blindness: A success story under Threat? *Plos Medicine* (3) 9: 1454-1460.

Boffill Càrdenas Marià., Sanchez Alvarez Carmen., Betancourt Morgado Emoe., Garcia Gutierrez Mariasabel., Lorenzo Monteaguolo Geidy., Sainz

Surrez Osaida., 2006. Toxicological Evaluation of an infusion of *Bidens pilosa*. *Pharmacologyonline*. 3: 428-434.

Bourguinat C., Pion D.S.S., Kamhno J., Gardon J., Duke B.OL., Boussinesq M., Prichard R.K., 2007. Genetic selection of low fertile *Onchocerca volvulus* by ivermectin treatment. *Neglected TropicalDiseases*. 1(1): 1-11.

Chia-Chi C., Ming-Hua Y., Hwei-Mei N., Jiing-Chuan C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*. 10 (3): 178-182.

Chinedu I., Kayode A. A., Chukwuma S. E., Ojochemeni E. Y., Chukwumaobin D. N., Nkiruka C. A., Yumanang G. S., 2019. Effects of ethanolic extracts of leaf, seed and fruit of *Datura mete* L. on kidney function of male rats. *Journal of Traditional and Complementary Medicine*. 9: 271-277.

Cho-Ngwa F., Abongwa M., Ngemenya M N., Nyongbela K D., 2010. Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca onchengi*. *BMC Complementary and Alternative Medicine*. 10: 62-69.

Cooper P.J., Awadzi K., Ottesen E.A., Remic D., Nutman T.B., 1999. Eosinophil sequestration and activation are associated with the onset and severity of systemic adverse reaction following the treatment of onchocerciasis. *Journal of Infectious Diseases*. 179: 738-742.

Coulibaly S.O., Ouattara A., Ouattara K., Coulibaly A., 2018. Toxicity studies of Aqueous and ethanolic extracts of fermented seeds of *Parkia biglobosa* (Mimosaceae) in rats. *International Journal of Sciences*. 7 (4): 40-45.

Cushnie T.P.T., Lamb A.J., 2005. Antibacterial activity of flavonoids. *International Journal of AntimicrobialAgents*. 26 (5): 343-356.

Cupp.E.W., Sauerbrey M., Richards F., 2011. Elimination of human onchocerciasis: History of progress and current feasibility using ivermectin (Mectizan) monotherapy. *Acta tropica*. 120 (1): 100-108.

Damien S.T.T., Koto-te-Nyiwa N., Lengbiye-E.M., Dorotheé D.T., Bienvenu M.M., Jeff B.I., Blaise M.M., Virima M., Pius T.M., 2016. Chemical composition and bioactivity of *Canariumschweinfurthii* bark extracts from DR Congo against Sickle Cell disease and associated bacteria. *Journal of pharmacognosy and phytochemistry*. 5 (4): 181-187.

Dedehou N.G., Olunladé P.A., Adenilé A.D., Azando E.V.B., Alowanou G., Daga F., Hounzangbé-Adoté M.S., 2014. Effet *in vitro* des feuilles de *Pterocarpus erinaceus* et des cosses de fruits de *Parkia biglobosa* sur deux stades du cycle

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Bursaceae) from the equatorial region (Cameroon).

de développement de *Haemonchus contortus* nematode parasite gastro-intestinal de petits ruminants. *Journal of Animal and Plant Sciences*. 22 (1): 3368-3378.

Dehpour A.A., Ibrahimzadeh M.A., Seyed F. N and Seyed M, 2009. Antioxydant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas y Aceites*. 60: 405-412.

Doligalska M., J ózwicka K., Kieslowski M., Mroczek A. P., Aczkowski. C., Janiszowska W., 2011. Triterpenoid saponins affect the function of P-glycoprotein and reduce the survival of the free-living stages of *Heligmosomoides bakeri*. *Veterinary Parasitology*. 179 : 144–151.

Ezekwe C.I., Ada C.A and Ugwu O.P.C., 2013. Effect of Methanol extract of *Parkia bigloboza* stem bark on the liver and kidney functions on the Albino rats. *Global Journal of Biotechnology and Biochemistry*. 8: (2): 40-50.

Fabricant D.S., Farnsworth N.R., 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. 109: 69-75.

Florencio M.S, and Peioxoto C.A, 2003. Effects of Diethylcarmazine on the ulstructure of microfilariae of *Wuchereria bancrofti*. *Parasitology*: 126: 551-554.

Galambos B., Csöngé L., Olah A., Von V. R., Tamas L., 2004. Quantitative reduction of Methyl Terazolium by fresh vein Hämograph biopsies *in vitro* is an index of viability. *European Surgical Research*. 36: 371-375.

Gardon J., Gardon-Wendel N., Nguangue D., Kamgno J., Chippau J.P., 1997. Serious reactions after mass treatment of onchocerciasis with ivermectin in arrea endemic for *Loa loa* infection. *Lancet*. 350: 18-22.

Hasanori A., Kazuyasu F., Osamu Y., Takashi D and Keiji I., 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*. 48: 487-491.

Hassan S.W., Umar A.A., Ebbo A.A., Akpeji A.J., Matazu I.K., 2008. Hepaprotective effect of leaf extracts of *Parkinsonia aculeate* L. against CCL intoxication in albino rats. *International Journal of Biochemistry*. 2 (2): 42-48.

Ihemeje A., Ojinnaka M.C., Obi K.C., Ekwe C.C., 2013. Biochemical evaluation of pepper fruit (*Dennettia tripetata*) and its use as substitute for giner in Zobo drink production. *Academic Research International*. 4: 513-521.

Illona G.F., Amy G.H., Yan S., Eugenia D., Helen F.M., Mark J., Eric P., 2006. Wolbachia and *Onchocerca volvulus* induced keratitis (River

Blindness) is depended on Myeloid differentiation factor 88. *American Society for Microbiology*. 74 (4): 2442-2445.

Irma P., Agnieska G., Danuta S, 2010. Saponins as cytotoxic agents: a review. *Phytochemistry Review*. 9: 425-474.

Jacob M.C., Favre M., Bensa J.C., 1991. Membrane cell permeabilization, with saponin and multiparametric analysis by flow cytometry. *Cytometry*. 12 (6): 550-558.

Kamtchouing P., Kahpui S.M., Djomeni P.D., Tedong L., Asongalem E.A., Dimo T., 2008. Anti-diabetic activity of methanol/methylene chloride stem bark extract of *Terminalia superba* and *Canarium shweinfurthhu* on streptozotocin-induced diabetic rats. *Journal of Ethnopharmacol*. 104 (3): 306-369.

Konan K.M., Mamyrbékova-Békro J.A., Békro Y.A., Djié B.M.G., Zomi B.T.J., 2011. *In vitro* antioxidant activities of total flavonoids extract from leaves and stems of *Adenia lobata* (Jacq). Eng (passifloraceae). *Journal of Pharmacognosy and phytotherapy*. 3(1): 8-12.

Li Y., Kui-Shan W., Xiao R., Ying-Xiang Z., Feng W and Qiang W., 2018. Response of plant secondary metabolites to environmental factors. *Metabolites*. 23 (762): 1-26.

Molan A.L., Sivakumaran S., Spenser P.A., Meagher L.P., 2004. Green tea flavan-ol and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagiacyrcumcinda* and *Trichostrongylus colubriformis* *in vitro*. *Research in Veterinary Science*. 77: 239-243.

Mueller-Harvey I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of food and Agriculture*. 86: 2010-2037.

Nathalie B., Jean-Paul C., 2006. Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. *Cahier des techniques de l'INRA*. 79-82.

Narayana R.K., Reddy S.M., Chaluvadi M.R., Krishna D.R., 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology*, 33: 2-16.

Ncube N.S., Afolayan A.J., Okoh A.I., 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and futurs trends. *African Journal of Biotechnology*. 7 (12): 1797-1806.

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

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

- Ndjonka D., Ayouba M., Ahamat Abakar., Djafsia B and Ndouwe T.M.H, 2017. *In vitro* toxicity study and antifilarial activity of four plants from Nord-Cameroon. *European Journal of Medicinal plants*. 19 (3): 1-12.
- Nenad M. Z., Milan S.S., 2017. Variability of secondary metabolites of the species of *Cichorium intybus* L. from different habitats plants. *Plants*. 2 (38) :1-19.
- N'Guessam K., Kadja B., Zirih G., Dossahoua G., Aké A. L., 2009. Screening phytochimique de quelques médicinales ivoiriennes utilisées en pays Kroou (Agbo-ville, Côte d'Ivoire). *Sciences et Natures* 6 (1) : 1-15.
- Noma M, Nwoke Bebe, Nutall I, Tamballa P.A, Enyong P, Namsenmo A, Remej, Amazigou U V, Kalle O O, Seketeli A., 2002. Rapid Epidemiological Mapping of Onchocerciasis (REMEO), its application by the African Program for Onchocerciasis Control. *Annals of Tropical Medicine and Parasitology*. 96: 29-39.
- Nnama T.N., Asomugha A.L., Asomugha R.N., Umeasalugo K.E and Mgbemena I.O., 2017. Phytochemical analysis and Acute toxicological study of *Erythrina senegalensis* ethanolic leaf extract in Albino Wistar rats. *Anatomy and Physiology*. 7(1): 248-250.
- Nwankpa P., Ekwegu C.N., Ekwurugwu J.N., Chukwuemeka O.G., Etteh C.C., Uguezumba P.C., Emengagah F.C., 2018. Assessment kidney function indices in male albino wistar rats administered ethanol stem bark of *Denndetia tripetala* (peper fruit). *Biochemistry and Pharmacology*. 7 (1): 242-245.
- Obase L.C., Koudou J., Kumulungui B.S., Bassole I.H., Edou P., Ouattara A.S., Traore A.S., 2007. Antioxidant and antimicrobial activities of *Canarium schweinfurthii* Engl. Essential oil from Central African Republic. *African Journal of Biotechnology*. 6 (20): 2319-2323.
- Ogukwe C.E., Oguzie E.E., Unaegbu C., Okolue B.N., 2004. Phytochemical screening on the leaves of *Sansevieria trifasciata*. *Journal of Chemical Society of Nigeria*. 29 (1) : 8-10.
- Okpekon T., Yolou S., Gleye C., Roblot F., Loiseau P., Bories C., Grellier P., Grellier P., Frappier F., Laurens A., Hocquemiller R., 2004. Antiparasitic activities of medicinal plants used in Ivory Coast. *Journal of Ethnopharmacology*. 9: 91-97.
- Okoli B.J., Ayo R.G., Ndukwe G., Ayo R.G., IHabila J.D., 2016. Inhibition of the developmental stages of *Ascaris suum* and anti-microbial activity of 3- β Hydroxylolean-12, 18-diene isolated from the aerial parts of *Canarium schweinfurthii* (Engl). *American Chemical Science Journal*. 11(3):1-11.
- Okwuosa C.N., Achukwu P.U., Nwachukwu D.C., Eze A.A., Azubuike N.C., 2009. Nephroprotective activity of stem bark extracts of *Canarium schweinfurthii* on Acetaminophen-induced renal injuries in rats. *International Journal of Medicine and Health Development*. 14 (1): 6-13.
- Omajate G.C., Enwa F.O., Ajewo A.O., Eze C., 2014. Mechanisms of antimicrobial actions phytochemicals against Enteric pathogens. A review. *Journal of Pharmaceutical, Chemical and Biological Sciences*. 2 (2): 77-85.
- Omura S., Crump A., 2004. The life and times of ivermectin. *Natural Review of Microbiology*. 2: 984-989.
- Onu A., Saidu Y., Ladan M.J., Bilbis L.S., Aliero A.A., 2013. Effect of aqueous stem bark extract of *Khaya senegalensis* on some biochemical, hematological and histopathological parameters of rats. *Journal of toxicology*. 9p.
- Organisation for Economic Co-operation and Development (OECD). Guidelines for testing of Chemicals. No 423, 2001.
- Osemwegie O.O., Dahunsi S.O., 2015. *In vitro* effects of aqueous and ethanolic extracts of *Parkia biglobosa* (Jacq.) on selected microorganisms. *Nigerian Journal of Biotechnology*. 29: 11-20.
- Patel J., Kumar G.S., Qureshi M.S., Jena P.K., 2010. Anthelmintic activity of ethanolic extract of whole plant of *Eupatorium odoratum*. *International Journal of Phytomedicine*. 2: 127-132.
- Prastan T., Bimlesh K., Mandeep K., Gurpreet K., Harleen K., 2011. Phytochemical screening and Extraction: A review. *International Pharmaceutica Scientia*. 1 (1): 98-106.
- Queslati S., Karray-Bouraoui N., Attia H., Rabhi M., Ksouri R., Lachaal M. Physiological and antioxidant responses of *Mentha pulegium* (Pennyroyal) to salt stress., 2010. *Acta Physiologicae Plantarum*. 32: 289-296.
- Rahman G., Seip H, 2007. "Bioactive forage and phytotherapy to cure and control endo-parasite diseases in sheep and goat farming systems. A review of current scientific knowledge". *Landbauforschung volkenrode*. 57 (3): 285-295.
- Ruyc., 1991. Advances in pharmacological studies of Silymarin., 1991. Memoria dos Instituto Oswaldo Cruz. 86: 79-85.
- Samje M., Metuge J., Mbah J., Nguesson B and Cho-Ngwa F., 2014. *In vitro* anti-Onchoceraochengi activities of extracts and chromatographic fractions of *Craterispermum laurinum* and *Morinda lucida*. *BMC Complementary and Alternative Medicine*. 14: 1-12.
- Sharma V., Chaudhary U., Singh R., Janmeda P., 2014. Evaluation of quantitative and antioxidant activity of *Achyranthes aspera* roots and inflorescences. *Asian Journal of Pharmaceutics*. 8: 54-60.

KOGA et al 2020: Anti-onchocercal activity and *in vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon).

Sokoujou J.B., Njateng G.S.S., Fodoup S.P.C., Kodjio N., Atsafack S.S., Fowa A.B., Dimeli M.N and Gatsing D., 2018. *In vitro* anti salmonellal activity and antioxidant activities of *Canarium schweinfurthii* stem bark extracts *Academia Journal of Medicinal plants*. 6 (110): 331-341.

Strauss S.K., Hancock R.E., 2006. Mode of action of new antibiotic for Gram-positive pathogens daptomycin, comparison with cationic antimicrobial peptides and lipopeptides. *Biochemica and Biophysica Acta*. 1758 (9): 1275-1223.

Taylor, M. J., A. Hoerauf, and M. Bockarie. 2010. Lymphatic filariasis and onchocerciasis. *Lancet*. 376 :1175–1185.

Verrest L., Dorlo T.P.C., 2017. Lack of clinical pharmacokinetic studies to optimize the treatment of neglected tropical diseases: a systematic review. *Clinical Pharmacokinetics*. 56 : 583-606.

Wabo Pone. J., Mpoawe.M. Bilong Bilong C.F., 2005. Etude comparative *in vitro* de l'activité nématocicide de l'extrait éthanolique de la poudre d'écorce de *Canthium mannii* (Rubiaceae) et du Mebendazole. *Revue de Médecine Vétérinaire*. 156 (12) : 633-636.

Wada H and Snell E.E., 1962. Enzymatic transamination of Pyridoxamine. II. Crystalline-pyruvate transaminase. *Journal of Biochemistry*. 237: 133-137.

Yakubu M.T., Bilibis L.B., Lawal M., Akanji M.A., 2003. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biochemistry*. 15: 50-56.

Yates D.M, 2003. The avermectin receptors of *Haemonchus contortus* and *Caenorhabditis elegans*. *International Journal of Parasitology*. 33 : 1183-1193.

Yaya.G, Kobangué.L, Kémata.B, Galli.D, Grésenguet.G. 2014. Elimination ou contrôle de l'onchocercose en Afrique ? Cas du village de Gami en République Centrafricaine. *Bulletin dela Société de Pathologie Exotique*. 107 :188-193.