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IN VITRO EFFECT OF *BURKEA AFRICANA* BURKE, 1840 (FABACEAE-CESALPINOIDEAE) ETHANOLIC BARK EXTRACT ON THE NEMATODE *HAEMONCHUS CONTORTUS* RUDOLPHI, 1803

Vroumsia Toua*, Adamou Ahmadou, Ndjonka Dieudonné
Department of Biological Sciences, Faculty of Science, University of Ngaoundere, PO Box 454 Ngaoundere, Cameroon.

Abstract:
In order to seek an alternative to conventional means of small ruminants heamonchosis treatment and valorise biodiversity, an acute toxicity test was conducted by incubating adult worms of Haemonchus contortus in a Phosphate Buffered Saline culture medium, containing increasing concentrations of Burkea africana trunk bark extracts. The positive control consisted of a levamisole solution, while the negative control consisted of a PBS solution containing DMSO. The anthelmintic activity, assessed every 6 hours for 24 hours, was expressed as a percentage of mortality. The data obtained show that, the ethanolic extract of the bark of Burkea africana display anthelmintic activity against Haemonchus contortus. The average values of 18h-LC_{50} were of 0.56 ± 0.10 mg/ml for the levamisole and 0.75 ± 0.09 mg/ml for the peel. After 24 hours, the mean LC_{50} values were of 0.09 ± 0.05 mg/ml, for peel and 0.27 ± 0.09 mg/ml for levamisole. The LC_{50} variance analysis, both after 18 and 24 h, shows that there is no significant difference (P> 0.05) between the anthelmintic activity of the ethanol extract of the bark and that of Levamisole. These results confirm the efficacy of the extract of Burkea africana, used in traditional medicine to treat diseases caused by nematodes. An acute toxicity test, performed on white mice (Mus musculus), revealed that Burkea africana ethanolic bark extract was not toxic at a dose of 2500 mg/kg body weight. Given these data, the ethanolic extract of Burkea africana bark could be envisaged as an alternative to conventional fight against small ruminant's haemonchosis.

Keywords: *Acute toxicity, anthelminthic activity, haemonchosis, lethal concentration, Mus musculus, plant extract.*

Corresponding author:
Vroumsia Toua,
Department of Biological Sciences,
Faculty of Science, University of Ngaoundere,
PO Box 454 Ngaoundere, Cameroon.
E-mail: tvroumsia@yahoo.fr.
Tel.: (+237) 677 81 00 57, (+237) 693 74 65 84.

QR code

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INTRODUCTION:

Small ruminants play an important role in the area of animal production and the whole economy of many developing countries [1]. Their breeding has numerous advantages: they are easy to handle because they do not require large surface area and they are not very demanding in forage quality [2].

Gastrointestinal strongyles are parasitic nematodes of sheeps and goats, constituting a major obstacle to the development of small ruminants, worldwide [3]. These small animals are infected after ingestion of L3 (stage 3 larvae) during the grazing [4]. Infestations of foraging animals are very common and lead to significant economic losses by mortality or decreased production of milk, meat and wool [5]. The haemonchosis in small ruminants is defined as a disease caused by a parasite of *Haemonchus* Genus that can reach animals of all ages and that is translated, essentially, by an anaemia syndrome whose evolution is rapidly fatal, in case of massive infestation [6]. Its geographical distribution is worldwide, because of its parasite large adaptation capacities to climate variations [7]. In tropical areas, 45% of young lamb mortality is due to this parasite [8].

In Africa, the prevalence of haemonchosis is of 90% in Niger and Kenya [9, 10]. It is of 75% in Cameroon [11, 12]. *Haemonchus contortus* is a blood-sucking worm located in the abomasum of small ruminants. Anaemia resulting from infestation generates metabolic disorders, leading to the non-economic value of the animal, or even death [6]. The socio-economic consequences of this infestation usually get diarrhoea, productivity declines, growth retardation, weight loss and lack of gain for the population of farmers [13]. Control methods against digestive strongyles are based on synthetic chemical treatments using anthelmintics. The used therapeutics are benzimidazoles and probenzimidazoles, imidazothiazoles, and macrocyclic lactones [14]. In general, these conventional anthelmintic molecules have a number of limitations: the presence of drug residues of these products in foodstuffs of animal origin, lethal effects contained in the faeces on living organisms and their limited utilization in organic farming [15]. There is, also, a total prohibition of the use of certain molecules in lactating females [16] and the costs are sometimes out of reach of rural farmers [17]. We can also note the appearance and development of parasites strains resistance [18].

Because of all these problems, which complicate haemonchosis control, it is necessary to find new drugs with a high anthelmintic activity to fight against the concerned parasitic nematode.

In Africa, over 80% of the population makes use of traditional medicine to deal with health problems [19]. The African continent is full of very diverse medicinal plants. Thus from the 300,000 plant species identified on the planet, 200,000 species live in tropical African countries and have various medicinal properties [20]. An ethnobotanical survey in the Northern region of Cameroon, followed by a laboratory screening, allowed us to retain *Burkea africana* to contribute to an additional drug delivery within the framework of the fight against small ruminants haemonchosis. This is a plant belonging to the Fabaceae-Cesalpiniodeae family whose therapeutic virtues, through the use of its bark, roots and leaves, have been proven in traditional medicine in Cameroon North Region, in the treatment of stomach pain, abscesses, oedema, epilepsy, bloody diarrhoea, etc. *Burkea africana* grows in woodland between 50-1750 meters of altitude, on light and well-drained soils, often on soft, sandy or gravelly red soils, but sometimes on the rocky hills or loam-clay soil [21].

The main objective of this work is to contribute to the search of a plant based treatment, with fewer side or no toxic effects and accessible to all, to fight effectively against the small ruminants haemonchosis.

MATERIAL AND METHODS:

Ethnobotanical survey

An ethnobotanical survey, including traditional practitioners, veterinarians and farmers was conducted in 3 Cameroonian localities, in order to identify medicinal plants used against intestinal worms. The survey forms contained information as the scientific and common names of plants, used parts, time of picking, harvest period, therapeutic indications, preparation techniques, doses, routes of administration, side effects, antidotes, etc.

Plant Material and chemicals

Sample collection

Samples of leaves, bark and fruits of different plants were harvested in April 2015, precisely in Ngong, (Cameroon North Region) and identified by Dr Tchobsala, Department of Biological Sciences, University of Ngaoundere, Cameroon. If not stated otherwise, all chemicals were purchased from Sigma (Deisenhofen, Germany).

Preparation of plants powder

The different parts of the plants (leaves, trunk barks, and fruits) were, after washing, dried in the shade for

20 days, crushed in a wooden mortar using a pestle and sieved through a fine mesh sieve of 0.4 mm diameter [22]. The powders of the extracts obtained were stored in glass jars with lid, away from light.

Preparation of the plants ethanolic extract

To prepare the ethanol extract, 50 g of dry matter from each part of the plants were weighted, using a balance (Kerm 440-45N) and put to macerate, in 500 ml of alcohol at 70 °C, for 48 hours at room temperature. This mixture was subsequently centrifuged (Centrifuge 5804- Eppendorf) at 3500 rpm, for 10 min, then the supernatant was collected and filtered (filter paper Blackribbon 5891). The filtrate is then passed to a rotary evaporator (Büchi, Switzerland) at 40 °C, for the concentration of the extract, then in an oven (Memmert) at 40 °C, to complete evaporation. The obtained products, as crystals for the barks extract and as a dough, for the fruits and leaves, were weighed, placed in sterile Falcon tubes and kept protected from light, and at 4 °C [23], for further use.

The yield (as a percentage) of extraction from the different plants parts was determined using the following formula:

$r = \left(\frac{m}{M}\right) x\, 100$, where r is the yield (%), m the mass of the obtained extract (g) and M the mass of the powdered part (leaves, trunk barks, and fruits) of the plant (g).

Preparation of plants ethanolic extracts stock solution

To prepare the stock solution of the ethanolic extract, 0.3 g of crystals or of dough were dissolved in 150 µl of 100% DMSO in a sterile Falcon tube. Thereafter, 2850 µl of distilled water were added. The mixture, at a concentration of 100 mg/ml, was homogenized (Vortex, Heidolph) and then protected from light and stored at 4 °C, for further use.

Animal material

Collection of worms

Samples of abomasum with worms, including *H. contortus*, of slaughtered sheeps and/or goats were harvested at the slaughterhouse of Bantaille Market in the town of Ngaoundere (Cameroon) and were brought to the laboratory. The abomasums were opened lengthwise along the greater curvature, using a scalpel blade. The worms present in the ingesta, or attached to the surface of the organ were harvested manually, using forceps, while those contained in the washing liquid of the walls were collected after a passage through a sieve of 0.212 µm of mesh (ASTME 11-81). The female worms were isolated by examination under an optic microscope (Wild Heerbrugg), then washed and suspended in PBS solution contained in a Petri dish.

Breeding and maintenance of mice

The mouse was obtained from the National Veterinary Laboratory (LANAVET, Garoua, Cameroon). With a body weight of 18 to 20 g, the 4 weeks old mice were placed in ventilated metal cages, containing regularly renewed wood chips litter. They were fed with cottonseed meal, millet flour, corn bran, palm oil, and water, and were acclimatized to the conditions of the laboratory animal house, for thirty days before treatment.

Acute toxicity tests *on Haemonchus contortus* adult females

Bark extracts screening of various plants (*Gardenia erubescens*, *Burkea africana* and *Vallis choudea*)

Plants from the ethnobotanical survey having already been the subject of previous studies have been eliminated from the screening. Only remained *Gardenia erubescens*, *Burkea africana* and *Vallis choudea*. A screening of bark extracts of these plants has been carried out to determine the most effective plant. According to the protocol of [24], 10 worms by concentration and one worm per well containing 500 µl of PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 1 l of distilled water) supplemented with the extracts, at increasing final concentrations of 1, 2 and 3 mg/ml, were incubated, at 37 °C in a CO_2 incubator, for 24 hours. After 24 hours, the worms were removed from the test media and the parasites suspended in PBS for 30 min for possible recovery of the parasite motility. Death of worms was ascertained by absence of motility for an observation period of 5-6 seconds [25]. The negative control wells contained DMSO at increasing concentrations of 0.05, 0.1 and 0.15%, corresponding to the respective extracts concentrations of 1, 2 and 3 mg/ml.

Different parts of *Burkea africana* (bark, leaves and fruits) ethanolic extract screening at 1 and 5 mg/ml.

A screening of different parts (bark, leaves, and fruits) of *Burkea africana* was conducted to determine the most effective part of the plant. According to the protocol of [24], 10 worms by concentration and one worm per well containing 500 µl of PBS supplemented with the extracts, at final concentrations of 1 and 5 mg/ml, were incubated, at 37 °C in a CO_2 incubator, for 24 hours. The negative control wells contained DMSO at concentrations of

0.05 and 0.25%, corresponding to the respective extracts concentrations of 1 and 5 mg/ml.

Acute toxicity test of the ethanolic extract of the bark of *Burkea africana*

After the screening of the different parts of *Burkea africana*, the barks have proven more efficiency, compared to the leaves and fruit. As a result of preliminary tests, a concentration range of 0 to 1 mg/ml was determined. Solutions of plant extracts were prepared with PBS at 6 different concentrations (0, 0.1, 0.3, 0.5, 0.7 and 1 mg/ml) and 1 ml of each of these solutions was deposited in the wells of culture plates. Active adult female worm, then was placed in each well. A negative (DMSO at 0.05%) and positive (levamisole obtained from a veterinarian local pharmacy) controls have also been prepared [26]. For each concentration, 10 worms were used and the test was repeated 3 times. The worms were incubated at 37 °C and mortality is determined at intervals of 6, 12, 18 and 24 hours by microscopic observation. The concentration that killed half of the worms (LC_{50}), after each time interval, was calculated based on the SPSS 16.0 software. For the positive control, levamisole solutions were prepared with PBS at the same concentrations as those of the plant extract.

Acute toxicity test on white mice *Mus musculus*

According to the protocol of [27], 3 g of plant extract were dissolved in 20 ml of distilled water to a final concentration of 150 mg/ml. From this stock solution, dilutions at 1/2, 1/3 and 1/4, corresponding to concentrations of 75, 50 and 37.5 mg/ml, respectively, were prepared. Before treatment, the animals were weighed (18-20 g) and fasted for 12 hours and then divided into 5 groups of 10 as follows: the first group is the control group and receives distilled water, the second to the fifth groups constituted the treated batches and receive the plant extract, at respective concentrations of 37.5, 50, 75 and 150 mg/ml, corresponding to doses of 625, 1225, 2500 and 5000 mg/kg. Using an oesophageal probe, the extract was administered in a single dose of 0.6 ml/20g of body weight. After feeding, the animals were observed immediately and every 30 minutes, for 8 hours the first day and once a day, for 48 hours. During this period, symptomatic disorders (agitation, lack of appetite, motor difficulties, and dyspnoea) were monitored. Dead animals were counted in each batch, during 48 hours. Percent mortality is calculated by the following formula:

$$\text{Percent mortality (\%)} = (\text{Number of dead mice} / \text{Total number of mice}) \times 100$$

Statistical Analysis

Data were analysed using SPSS 16.0 software. Lethal concentrations (LC_{50}) at different time slots were determined by the SPSS 16.0 software. Z test was used to compare the mortality percentage means, whereas analysis of variance (ANOVA) was used to compare the averages of the LC_{50}.

RESULTS AND DISCUSSION:

Ethanolic extraction of *Burkea africana* bark yield

After evaporation, we obtained 11.30 g of ethanolic extracts of *Burkea africana* bark, from the 50 g of initial used powder, so a yield of 22.60%. Generally, extraction yields vary with the extracted part of the plant and with the utilized solvent. For instance, [26] had conducted hydromethanolic extraction of leaves of *Pterocarpus erinaceus* and cloves of *Parkia biglobossa* and obtained yields of 11 and 30.20, respectively.

Screening

Effect of *Gardenia erubescens*, *Burkea africana* and *Vallis choudea* ethanolic barks extracts on *Haemonchus contortus* adult female

From 0 to 6 hours, there was no mortality, at 1 mg/l of ethanolic extract of the bark of each of these 3 plants. At the 6-24 hours interval, mortality rate increased gradually, for the three extracts. After 24 hours, it reached the mean values of 50, 72 and 67 % for *Gardenia erubescens*, *Burkea africana* and *Vallis choudea*, respectively (Fig. 1A).

At 2 mg/ml of the ethanolic extract of the bark of each of the plants, there was no mortality, after 6 hours of incubation. The percentage of mortality, then, gradually, increased. Between 12 and 18 hours of incubation, the mortality rate was almost constant for *Vallis choudea* and grew faster for the two others. At 24 hours, it was respectively of 72, 83 and 44% of dead worms, for *Gardenia erubescens*, *Burkea africana* and *Vallis choudea* (Fig. 1B). There is a statically significant difference between the activity of *Gardenia erubescens* and that of *Vallis choudea* ($|Z| > 1.96$) and between that of *Burkea africana* and that of *Vallis choudea* ($|Z| > 1.96$).

At 3 mg/ml, mortality rate was of 0%, 6 hours after incubation (Fig. 1C). The percentage of mortality, then, increased progressively. At 18 hours of incubation, there was a high mortality of worms (83%), for *Burkea africana*. After 24 hours of incubation, we had 89, 95 and 39% of dead worms, respectively, for *Gardenia erubescens*, *Burkea africana* and *Vallis choudea* (Fig. 1C). There is a statically significant difference between the activity of *Gardenia erubescens* and that of *Vallis choudea* ($|Z| > 1.96$) and between that of *Burkea africana* and that of *Vallis choudea* ($|Z| > 1.96$).

From these results, *Burkea africana* seemed to be more efficient than *Gardenia erubescens* and *Vallis choudea*. So, the ethanolic extract of the bark of *Burkea africana* revealed a higher anthelmintic effect on *Haemonchus contortus* adult female than those of *Gardenia erubescens* and *Vallis choudea*, globally. The extracts we obtained seem to be more efficient than those used by [28] who had found that *Eclipta prostrata* methanolic whole plant extracts had *Haemonchus contortus* mean mortality of 40.0 % at 6.25 mg/ml, 53.3 % at 12.5 mg/ml and 56.7% at 25 mg/ml, after 24 hours.

Effect of different parts (bark, leaves, fruits) of *Burkea africana*

At 1 mg/ml and after 24 hours, the ethanolic extracts of the different parts of *Burkea africana* displayed anthelmintic activity of 100, 90 and 80% of *Haemonchus contortus* adult female mortality, for barks, leaves and fruits, respectively (Fig. 2A). There is a statically significant difference between the activity of *barks* and that of fruits ($|Z| > 1.96$). At 5 mg/l and after 24 hours, the 3 plants parts had the same amount of activity (mortality average values of 100 %, Fig. 2B).

Evaluation of the anthelmintic effect of *Burkea africana* bark

A survival test of the worms, aiming to determine the optimum lifetime of *Haemonchus contortus* adult female in a PBS culture medium, showed that these worms have a 100% survival rate until 24 hours of incubation. This result profile is similar to that obtained by [26], who, in their study of the anthelmintic effect of acetonic and methanolic extracts of *Pterocarpus erinaceus* and *Parkia biglobosa* on *Haemonchus contortus* adult worms motility, got 100% of mobile worms after 24 hours, in PBS. Otherwise, incubation of *Haemonchus contortus* adult females in the presence of 0.1% DMSO, for 24 hours resulted in no mortality. These results are similar to those obtained by [23], concluding that the low concentrations (1%) of DMSO present in the PBS solution does not affect the survival of the worm *Onchocerca ochangi*. This implies that the effects of the tested solutions on the worms are due exclusively to our extracts. This result, thus, validates the PBS medium as test medium.

The nematotoxic effects of *Burkea africana* barks, resulting in the mortality of *Haemonchus contortus* adult female in PBS culture medium, vary with the test concentration and with the incubation time (Fig. 2C). At the 0-6 hours interval, the mortality rate was 0%. From 12 to 18 hours, the mortality mean varied from 17 to 50 %, according to the different test concentrations. After 24 hours, a mortality rate of 94%, at 1 mg/ml of *Burkea africana* bark extract, was recorded. [29], studying *in-vitro* anthelmintic effects of two Kenyan plant extracts against *Heamonchus contortus* adult worms, have found that roots bark of *Entada leptostachya* and stems bark of *Raponea rhododendroides* methanolic extracts, at 25 mg/ml each, were more potent against the parasites, with mortalities of 77 and 54%, after 24 hours. It appear that *Burkea africana* bark extract, in our experience conditions, is more efficient than the plants extracts these authors used. The same conclusion can be set, when considering the results of [30], who had reported that methanolic roots extract of *Vernonia amygdalina* gave *H. contortus* adult worms mortality of 33.3 %, at 6.25 mg/ml, 46.7%, at 12.5 mg/ml and 56.7%, at 25 mg/ml, after 24 hours.

The ethanolic extract of *Burkea africana* bark showed anthelmintic *in vitro* effect on *Haemonchus contortus* adult females, at different incubation times and at 37 °C. Averages LC_{50} of 0.75 ± 0.09 mg/ml at 18 hours and of 0.09 ± 0.05 mg/ml at 24 hours, were obtained (Table 1), during this work. Somewhere else, [31] had found that the 24 hours lethal concentrations (LC_{50}-24H) against *H. contortus* were 0.8 ± 0.4, 0.39 ± 0.4, 0.17 ± 0.1 and 1.04 ± 0.7 mg/mL for the roots barks aqueous extract of *Cassia sieberiana*, the leaves aqueous extracts of *Cassia sieberiana*, of *Guiera senegalensis* and of *Sapium grahamii*, respectively. Beyong these values, the lowest one that can be brought closer to the 24H-LC_{50} we obtained is that of the leaves aqueous extracts of *Guiera senegalensis*. It must be noted that leaves aqueous extracts of various plants seem to be efficient against *H. contortus*. For instance, [32] had demonstrated that aqueous extracts of leaves of *Carissa spinarum* and *Azadrichta indica* showed very good activity against the adult worms of *H. contortus*, mortality raised to the levels of 96.8 and 93.9%, respectively, at concentration of 4 mg/ml, after 24 hours.[33] had revealed that out of 7 plants, *Calotorpis procera* methanolic leaves extracts was very vigorous against adult worms of *Haemonchus contortus*, after 1 hour, with LC_{50} value of 11.30 mg/ml, and of 7.94 and 3.44 mg/ml, after 2 and 3 hours, respectively. To explain the great variation in all these results, it can be said that, apart the difference in species, experimental conditions, plant age, habitat and part used, harvest season could also contribute to the variation in biochemical profiles and yields [34]. [35] , studying the antidiarrheal activity of the ethanolic extract of *Burkea africana* bark, showed that the extract fight against diarrhoea and that this effect could be due to inhibition of the biosynthesis in nematodes

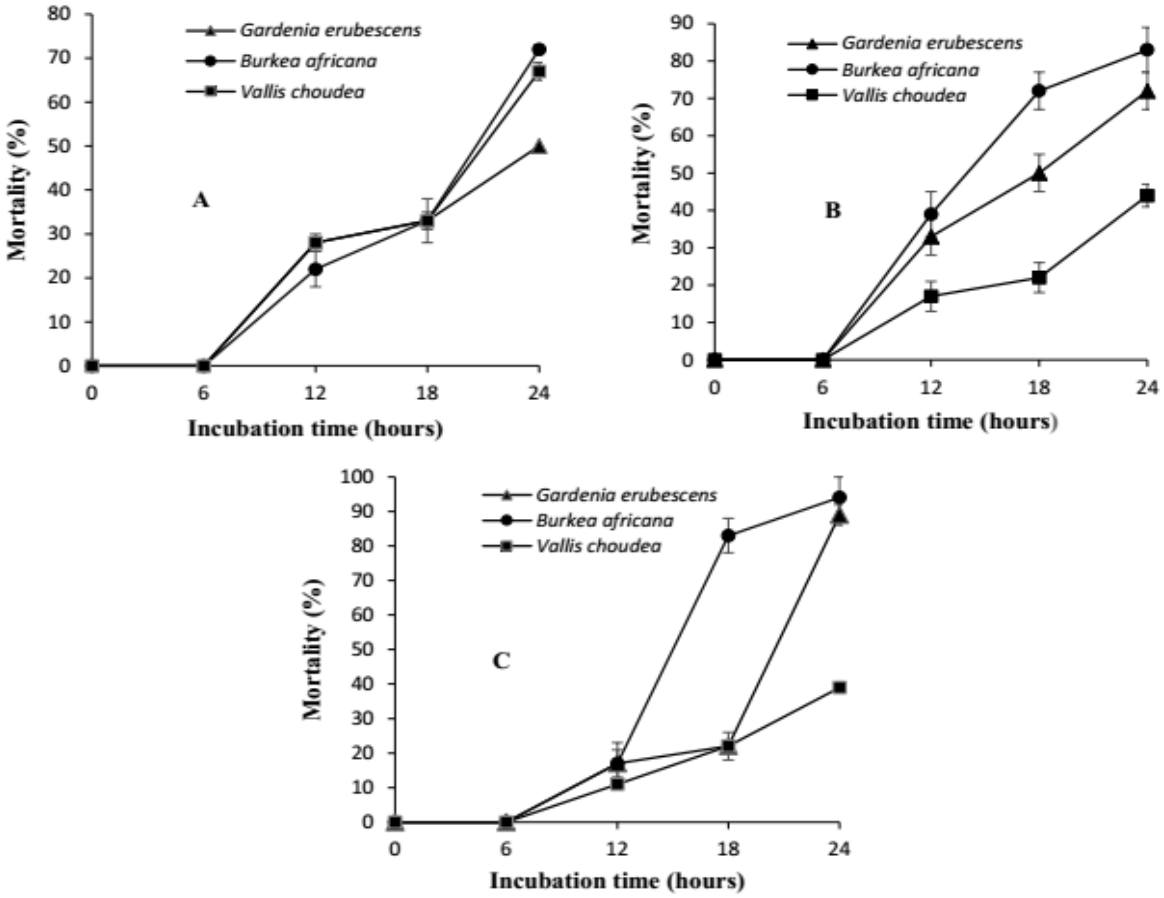

Fig 1: Mortality of Haemonchus contortus adult female in PBS medium containing ethanolic trunk bark extract of Gardenia erubescens, Burkea africana and Vallis choudea, at 1 (A), 2 (B) and 3 mg/L (C), along the time.

Nematotoxic effect of levamisole on *Haemonchus contortus* adult female

The effects of levamisole, at different concentrations, on the mortality of *Haemonchus contortus* in PBS culture medium are shown in figure 2D. It appears that the drug begins to act since the 0.5 mg/ml concentration, at 12 hours and at 0.7 mg/ml after 6 hours. At 18 hours, a gradual mortality is noted as a function of concentration. At 24 hours of incubation, 100% of mortality was recorded at 1 mg/ml. So, anthelmintic activity of levamisole has been confirmed.

From imidazothiazoles family, levamisole is an agonist of the nicotinic receptors responsible of nematodes muscle hypercontraction. The death of these ones would result from the prolonged stimulation of nicotinic receptors of the muscles. Moreover, this molecule has no effect on the paralyzed larva [36, 37]. It is therefore possible that the active ingredients present in our various extracts have acted as acetylcholinesterase inhibitor, leading to paralysis. The mean LC_{50} obtained were of 0.56 ± 0.10 mg/ml at 18 hours and 0.27 ± 0.09 mg/ml at 24 hours (Table 1).

Previous works on phytochemical screening of *Burkea Africana* bark had revealed the presence of many chemical families as flavonoids, tannins, saponins, cardiac glycosides, anthraquinones, triterpenes, trypsins and sterols [33, 38, 39]. Generally, the anthelmintic effect of *Burkea Africana* bark is attributed to these chemicals [39, 40]. Moreover, tannins are reported to cause anthelmintic activity by binding to the free proteins found in the gastrointestinal tract of the host animal or to glycoproteins on the cuticle of the parasite and may cause death [41].

It seems that bark extract acts slowly on *Haemonchus contortus* female adult, along the time, compared to levamisole. It is noted at 18 hours, a mean LC_{50} value of 0.75 ± 0.09 mg/ml, for peel and of 0.56 ± 0.10 mg/ml, for levamisole and at 24 hours, we had a

mean LC_{50} of 0.09 ± 0.05 mg/ml for bark and of 0.27 ± 0.09 mg/ml, for levamisole. The analysis of variance (ANOVA) showed that there was no significant difference (P > 0.05) between the effect of levamisole at 18 hours and that of the bark at 18 hours, and between that of levamisole at 24 hours and that of bark at 24 hours. This means that the ethanolic extract of the bark of the plant would be almost as effective as the reference medicinal product which is levamisole.

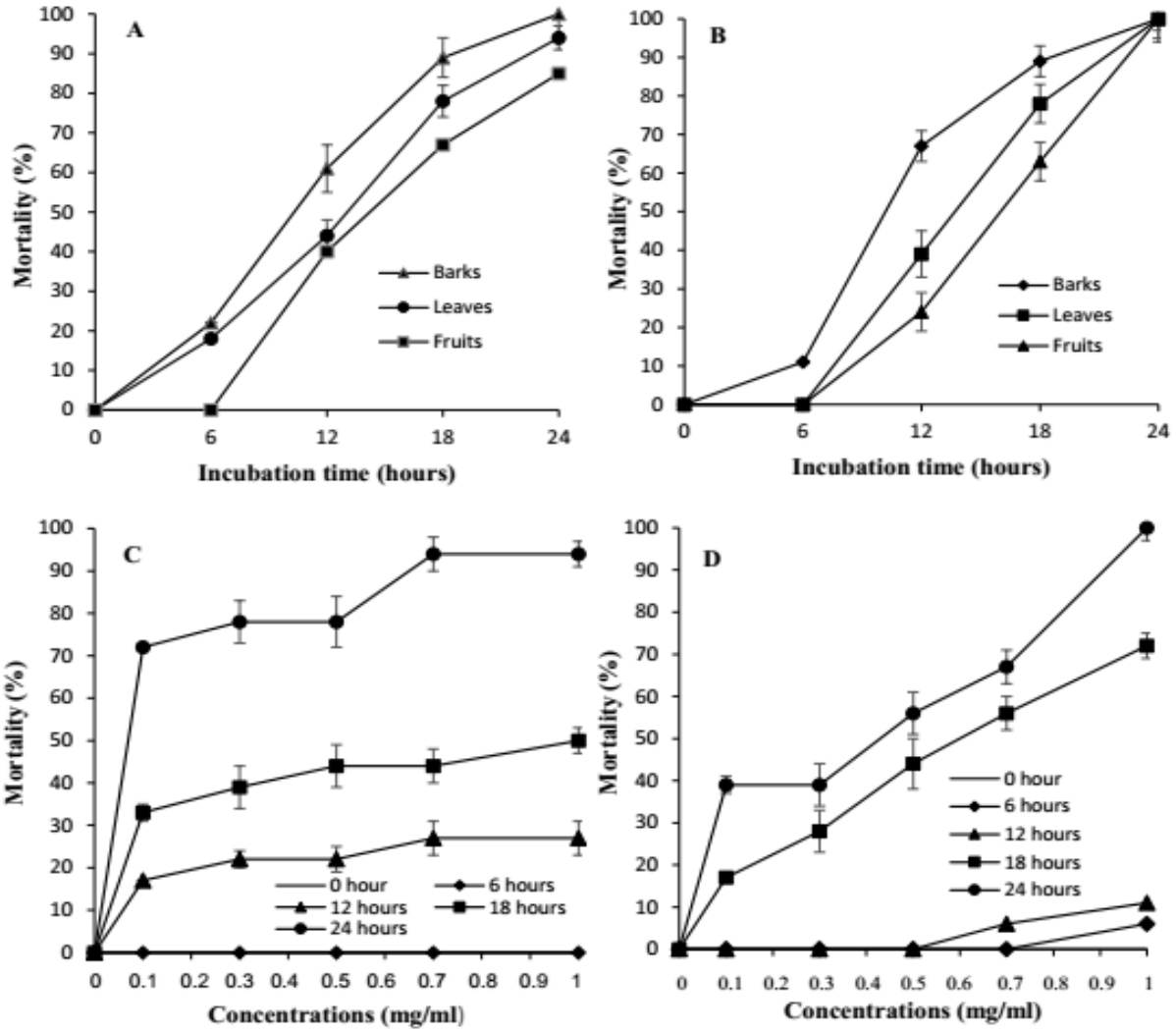

Fig 2: Mortality of *Haemonchus contortus* adult female, in PBS medium containing stem bark, fruits and leaves ethanolic extracts of *Burkea africana*, at 1 (A) and 5 mg/L (B), along the time and in PBS medium containing ethanolic stem bark extracts of *Burkea africana* (C) and levamisole (D) at increasing concentrations.

Table 1: *Burkea africana* ethanolic trunk bark extract and levamisole LC_{50} mean

	LC_{50} mean	
Incubation time (hours)	**Burkea africana ethanolic stem bark extract**	**Levamisole**
12	1.278 ± 0.09	ND
18	0.754 ± 0.09	0.56 ± 0.10
24	0.093 ± 0.05	0.27 ± 0.09

ND: Not determined

Clinical signs of mice forced-feeding with the *Burkea africana* extract

After forced-feeding of mice with *Burkea africana* trunk bark extract, at doses ranging from 625 to 5000 mg/kg of body weight, symptomatic disorders were observed in groups 2 and 3: drowsiness, lack of appetite, motor difficulties, dyspnoea, and withdrawal. A short agitation period of 27 seconds was also noted.

In groups 4 and 5, there were the same symptoms, with the addition of mice aggressiveness. Two hours later, all animals regained their normal habit. Changes regarding the general appearance of the mice were also noted at the hair (tight hair) and eyes (pronounced redness).

This experiment shows that the extract of the plant seems to have, at various doses, a stressful effect on mice.

Effect of forced-feeding on mice mortality

The extract was administered orally by single force-feeding. The administration of the extract at a dose of 5000 mg/kg of body weight, caused the death of 3 mice on 10, so a mortality of 30 %, after 24 hours (Table 2). Therefore, the LD_{50} of the ethanolic extract of *Burkea africana* trunk bark is greater than 5000 mg/kg. Likewise, [32] had reported that, by oral route, aqueous extract from 3 plants (*Cassa sieberiana, Guiera senegalensis and Sapium grahamii*) did not present toxicity on mice up to 2000 mg/kg of body weight.

According to the toxicity scale of [42] and [43], the ethanolic extract of *Burkea africana* trunk bark is not toxic to the white mouse *Mus musculus*.

Table 2: Mice mortality, at 24 hours after forced-feeding with increasing doses of ethanolic trunk bark extract of *Burkea africana*.

Mice group	Control	1	2	3	4
Forced feeding substance	Distilled water	Trunk bark extract	Trunk bark extract	Trunk bark extract	Trunk bark extract
Dose (mg/kg wb/or)	30 ml/kg	625	1225	2500	5000
Tested mice	10	10	10	10	10
Dead mice	0	0	0	0	3
Mortality (%)	0	0	0	0	30

CONCLUSION:

We have assessed, *in vitro*, the effect of the ethanolic extract of the trunk bark of *Burkea africana* on the parasitic nematode *Haemonchus contortus*. Ultimately the trunk bark showed a nematicid *in vitro* activity on the parasitic nematode. The LC_{50} obtained are of 0.75 ± 0.09 mg/ml at 18 hours and of 0.09 ± 0.05 mg/ml at 24 hours, for bark and of 0.27 ± 0.09 mg/ml, after 24 hours, for levamisole, the reference medicinal product. Thus, the ethanolic extract of the bark is an effective anthelmintic. Despite some clinical signs during the acute toxicity test on mice, the DL_{50} was estimated to exceed 5000 mg/kg. The ethanolic extract of *Burkea africana* anthelmintic activity was almost equivalent to that of the reference product which is levamisole. This plant is not toxic at a dose of 2500 mg/kg and is as active as levamisole, the reference drug, so it could be used to fight against small ruminants haemonchosis, worldwide.

For the future, it is planned to make qualitative and quantitative phytochemical tests of the plant.

REFERENCES :

1.Killanga S, Traoré A. Influence de la gestion et du statut socio-économique de l'agro-pasteur sur la productivité des ovins et des caprins au Mali central. Tropicultura, 1999; 4: 180-183.

2.Tchoumboué J. 1997. Elevage des caprins en zone soudano-guinéenne d'altitude du Cameroun: Visite d'étude sur les systèmes d'élevage dans les zones humides et subhumides d'Afrique. Wageningen, Allemagne: CTA.

3.Chartier C, Horst I. Efficacy of four broad spectrum anthelmintics against gastrointestinal nematodes in goat. Vet Res, 1994; 134: 523-524.

4.Brunet S. 2008. Analyse des mécanismes d'action antiparasitaire de plantes riches en substances polyphénoliques sur les nématodes du tube digestif des ruminants. Thèse de Doctorat de l'Université de Toulouse, Toulouse, France.

5.Berrag B, Cabaret J Gastro-intestinal and pulmonary nematode infections decrease goat productivity in Morocan semi-arid condition. J

Helminthol, 1998; 72: 15-20.

6.Chermette R. L'haemonchose ovine et ses particularités: importance et situation actuelle en France. Point Vét, 1982; 13: 21-28.
77.Waller PJ. Anthelmintic resistance. Vet Parasitol, 1997; 72: 391-405.
8.Vlassoff A, Mckenna PB. Nematode parasites of economic importance in sheep in New Zealand. New Zealand J Zool, 1994; 21: 1-8.
9.Nwosu CO, Ogunrinal AF, Fagbemi BO. Prevalence and seasonal changes in the gastro-intestinal helminths of Nigerian goats. J Helminthol, 1996; 70: 329-333.
10.Gatongi PM, Prichard, RK, Ranjan S, Gathuma JM, Munyua WK, Cheruyot H, Scott ME. Hypobiosis of *Haemonchus contortus* in natural infections of sheep and goats in a semi-arid area of Kenya. Vet Parasitol, 1998; 77: 49–61.
11.Ndamukong KJN, Sewell MMH, Asanji MF. Disease and mortality in small ruminants in the North West Province of Cameroon. Trop Ani Heal Prod, 1989, 21: 191-196.
12.Adjanohoun JE, Aboubakar N, Dramare K, Ebo ME, Ekpere JA, Enoworock E, Focho D, Gbile ZO, Kamanyi A, Kamsukom J, Keila NK, Nancy WL, Nkongmeneck B, Sofowara A, Tamze V, Wirmum CK. 1996. Contribution to ethnobotanical and floristic studies in Cameroon. Yaoundé, Cameroun: CSTR/OUA.
13.Tabel J, Sauve C, Cortet J, Tournadre H, Thomas Y, Cabaret J. Fonder l'évaluation de la thérapeutique sur l'individu ou sur le groupe ? Un exemple: homéopathie et strongles digestifs des ovins. Inn Agron, 2009; 4: 61-65.
14.McKellar QA, Jackson F. Veterinary anthelmintics: old and new. Trends Parasitol, 2004; 20: 456-61.
15.Cabaret J, Bouilhol M, Mage C. Managing helminths of ruminants in organic farming. Vet Res, 2002; 33: 625-640.
16.Chartier C, Hoste, H. La thérapeutique anthelminthique chez les caprins. Point Vét, 1997; 28: 125-132.
17.Tamboura H, Kaboré H, Yaméogo SM. Ethnomédecine vétérinaire et pharmacopée traditionnelle dans le plateau central du Burkina Faso : cas de la province du Passoré. Biotechnol Agron Soc Environ, 1998; 2: 181-191.
18.Okombe VE, Mbumba T, Pongombo CS. Efficacité antiparasitaire de la poudre de graines de courge (*Cucurbita moschata* L.) sur les helminthes gastro-intestinaux de la chèvre locale élevée à Lumumbashi en République Démocratique du Congo. Int J Biol Chem Sci, 2013; 7: 953-960.
19.WHO (Word Health Organization). 2000. Onchocerciasis (river blindness). [ONLINE]. Available at http: // www.who.int/mediacentre/factsheets/fs095/en/. [Accessed 15 January 2016].
20.Sofowora A. 1993. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Limited.
21.Arbonnier M. 2004. Trees, shrubs and lianas of West African dry zones. Montpellier, France: CIRAD.
22. Mensah JK, Ihenyen J, Iyamu M. Phytochemical and antimicrobial properties of *Emilia coccinea* (Cass). Asian J Cont Sci, 2013; 2: 26-31.
23.Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achuki D, Nukenine EN, Hensel A, Liebeu E. *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. J Helminthol, 2011; 85: 304-312.
24.Borsboom GJJM, Boatin BA, Ngelkerke NJ, Agoua H, Akpoboua KL, Alley EW, Bissan Y, Renz A, Yameogo L, Remme JH, Hbbema JD. Impact of Ivermectin on onchocerciasis transmission: assessing the empirical evidence that repeated Ivermectin mass treatments may lead to elimination/eradication in West-Africa. Filaria J, 2003; 2: 1- 25.
25.Rabel B, McGregor R, Douch PGC. Improved bioassay for estimate of inhibitory effects of ovine gastrointestinal mucus and anthelmintic on Nematodes larval Migration. Int J Parasitol, 1994; 24: 671- 676.
26.Dedehou VFGN, Olounladé PA, Adenilé AD, Azando EVB. Effets *in vitro* des feuilles de *Pterocarpus erinaceus* et des cosses de fruits de *Parkia biglobosa* sur deux stades du cycle de développement de *Haemonchus contortus*, nématode parasite gastro-intestinal des petits ruminants. J Ani Pl Sci, 2014, 22: 3368-3378.
27.Aké-Assi E, N'Guessan K et Kouassi AF. Evaluation de la toxicité aiguë de l'extrait aqueux des feuilles de *Thunbergia atacorensis*, une espèce nouvelle. Eur Sc J, 2015; 11: 92-100.
28.Sirama V, Kokwaro J, Owuor B, Yusuf A. *In-Vitro* Anthelmintic bioactivity study of *Eclipta prostrata* L. (Whole Plant) using adult *Haemonchus contortus* worms. A case study of Migori County, Kenya. *IOSR* J Pharm Biol Sci, 2014; 9: 45-53.
29.Ombasa O, Kareru PG, Rukunga G, Mbaria J, Keriko JM, Njonge FK, Owuor BO. *In-vitro* anthelmintic effects of two Kenyan plant extracts against *Heamonchus contortus* adult worms. Int J Pharmacol Res, 2012; 2: 113-116.
30.Sirama V, Kokwaro J, Owuor B, Yusuf A, Kodhiambo M. *In-Vitro* Anthelmintic activity study of *Vernonia amygdalina* Del. (asteraceae*)* roots

using adult *Haemonchus contortus* worms. Int J Pharm Res, 2015; 5: 1-7.
31.Traoré A, Ouedraogoa S, Kaboreb A, Tamboura HH, Guissou IP. The acute toxicity in mice and the *in vitro* anthelminthic effects on *Haemonchus contortus* of the extracts from three plants (*Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii*) used in traditional medicine in Burkina Faso. Ann Biol Res, 2014; 5: 41-46.
32. Mohammed A, Abebe Wossene A, Mirutse Giday M, Getachew Tilahun G, Nigatu Kebede N. *In vitro* anthelminthic activities of four medicinal plants against *Haemonchus contortus.* Afr. J. Pl Sci, 2013; 7: 369-373.
33.Yadav P, Kumar A, Vihan VS, Mahour K, *In vitro* adulticidal screening of various plant extract against adult *Haemonchus contortus.* J Pharm Res, 2009; 2: 1262-1263.
34.Habtemariam S, Gray AI, Waterman PG. Diterpenes from the leaves of *Leonotis ocymifolia* var. *raineriana*. J Nat Prod, 1994; 57: 1570-1574.
35.Galvez J, Crespo ME, Jimenez J, Suarez A, Zarzuelo A. Antidiarrhoeic activity of quercitrin in mice and rats. J Ethnopharmacol, 1993; 45: 157-159.
36.Martin RJ, Murray I, Robertson AP, Bjorn H, Sangster N. Anthelmintic and ion-channels: after a puncture, use a patch. Int J Parasitol, 1998; 28: 849-862.
37.Kohler P. The biochemical basis of anthelmintic action and resistance. Int J Parasitol, 2001; 31: 336-345.
38.Knowles CH, Martin JE. Slow transit constipation: a model of human gut dysmotility. Review of possible aetiologies. Neurogastroenterol Mot, 2000; 12: 181-96.
39.Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterol, 2006; 130: 1480-1491.
40.Wadré S, Kaboré A, Bayala B, Traoré A, Tamboura HH, Belem AMG. *In Vitro* comparative anthelmintic activity on *Hæmonchus contortus* of two natural fodders (*Cassia obtusifolia* and *Piliostigma reticulatum*) extracts used in Burkina Faso. Int J Agri For, 2015; 5: 146-150.
41.Kane SR, Mohite SK, Rajarambapu JSS. Anthelmintic activity of aqueous and methanol extracts of *Euphorbia tymifolia*. Int J Pharm Tech Res, 2009; 1: 666-669.
42.Hodge HC, Stemer JH. 1943. Determination of substances acute toxicity by LDB50B. American Industrial Hygiène Association.
43.Diezi J. 1989. Toxicology: Basic principles and chemical impact. In: Slatkine M, ed. Pharmacology: Fundamental Principles and Pratice. Geneva: Academic Press 33-44.