

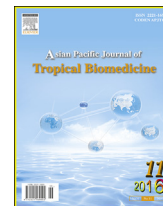
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journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)Original article <http://dx.doi.org/10.1016/j.apjtb.2016.07.015>Anthelmintic activity of *Saba senegalensis* (A.DC.) Pichon (Apocynaceae) extract against adult worms and eggs of *Haemonchus contortus*

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## ABSTRACT

**Objective:** To evaluate the anthelmintic property of *Saba senegalensis* (A.DC) Pichon (Apocynaceae) (*S. senegalensis*) on *Haemonchus contortus* that is traditionally used in Burkina Faso for its gastrointestinal parasites treatment.

**Methods:** The lyophilized aqueous decoction of leaves of *S. senegalensis* at concentrations of 0.10, 1.00, 3.00, 10.00 and 15.00 mg/mL was used on eggs and adult worms of *Haemonchus contortus* collected from gastrointestinal tract of small ruminant.

**Results:** The LC<sub>50</sub> on adult worms was 6.79 mg/mL and 3.25 mg/mL for the leaves of *S. senegalensis* and the levamisole (reference drug), respectively. Inhibition of hatching assay showed a concentration-dependent manner with an inhibition of 93.63% at the concentration of 15.00 mg/mL of *S. senegalensis*.

**Conclusions:** These results indicate that the aqueous extract of *S. senegalensis* possesses an anthelmintic property and may justify its use in traditional medicine for the treatment of gastrointestinal parasites.

## 1. Introduction

Neglected tropical diseases affect more than one billion people, mostly living in hot climates [1]. They represent a major public health and economic importance to both man and animals throughout the tropics and especially in developing countries.

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The tests in this work were performed according to the protocol approved by the ethical statement of the committee of Institute for Research in Health Sciences (IRSS).

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Nowadays, the parasitic diseases constitute a growing and frequent human and animal health stress by causing serious economic losses in livestock farming [2]. In fact, they are responsible for huge economic losses, cause stunting of individuals and their long-term effects induce debilitating nature of pathology [1]. In parasitized hosts, the presence of worms is mostly related by gastrointestinal disorders (diarrhea, abdominal pain), respiratory disorders (cough), etc. In addition to these ailments, we note most of the time, severe anemia and a deficiency of vitamins A [1]. In tropical regions, helminthiasis represent the most common parasitic illness [1]. The main strategy of prevention is based on the practice of daily hygiene, nutrition and the use of anthelmintic drugs quite expensive for the rural populations [1]. In addition to the importance of economic losses caused by these diseases, the use of anthelmintic drugs led to the development of resistance to available molecules [3].

Thus, the search for more effective and accessible drugs through traditional medicine is necessary to fight against these intestinal parasites. Like other developing countries, plants are widely used in traditional medicine for the treatment of various human and animal diseases such as parasitosis [4]. Antibacterial and antiparasitic activities of decoctions of the leaves of *Saba senegalensis* (A.DC) Pichon (*S. senegalensis*) were reported in Guinea, Burkina Faso and Ivory Coast following ethnobotanical surveys respectively by Magassouba *et al.* [5], Traore *et al.* [6], Koné and Atindehou [7], which indicated that these plants would have antiparasitic property against gastrointestinal parasites.

The leaves of *Azadirachta indica* and stem barks extract of *Bridelia ferruginea* are used against gastrointestinal nematodes [8,9]. The crude aqueous extracts of the leaves of *Carissa spinarum*, *Azadirachta indica*, *Myrsine africana* and *Rhus glabrous* indicate potential anthelmintic effect on *Haemonchus contortus* (*H. contortus*) [10,11]. Moreover, *in vitro* activities of acetonic extracts from leaves of three forage legumes (*Calliandra calothyrsus*, *Gliricidia sepium* and *Leucaena diversifolia*) on *H. contortus* revealed an anthelmintic activity on the same parasite [12] while the methanolic extract of flower of *Mallotus philippensis* possessed potent anthelmintic property against the third stage larvae of *H. contortus* [13].

In Burkina Faso, more than 427 plant species were used by the Mossi populations for the treatment of various diseases including parasites but their scientific results were not all documented [4]. Several studies showed anthelmintic properties of medicinal herbs such as the extracts of *Anogeissus leiocarpus* and *Daniellia oliveri* that indicate some effects on the eggs and adult worms of *H. contortus* [14]. Likely, extracts of *Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii* were used in Burkina Faso traditional medicine for their anthelmintic effect on adult worms and eggs of *H. contortus* [15]. In this study, we evaluate the anthelmintic property of *S. senegalensis* (Apocynaceae) on *H. contortus* that is traditionally used in Burkina Faso for its gastrointestinal parasites treatment.

## 2. Materials and methods

### 2.1. Plant material

Leaves of *S. senegalensis* were collected around Bassinko district about 30 km at the north of Ouagadougou, in July 2012. A sample of the plant was identified by plant taxonomist at the herbarium of the National Center for Scientific and Technological Research and a voucher specimen was deposited under No. 00223 HNBU. The leaves of plants were air dried at room temperature, powdered using pestle and mortar and kept in amber colored bottle until use in order to keep all their physicochemical properties.

### 2.2. Plant extract

Extraction was conducted at the chemical laboratory of the Department of Medicine and Traditional Pharmacopoeia – Pharmacy at the Institute for Research in Health Sciences. A decoction of *S. senegalensis* was prepared by soaking a weighed amount of the dry powder (50 g) in distilled water (500 mL) and the mixture was boiled for 45 min. After freeze, the decoction obtained was filtered through a nylon cloth and then centrifuged

at 2000 r/min for 5 min. The supernatant was collected and a portion was concentrated in an oven at 50 °C for 24 h, congealed and then lyophilized. The lyophilized dry powder was then collected in a stoppered sample vial, weighed and kept in a desiccator to avoid absorption of water until use in the assay.

### 2.3. *In vitro* anthelmintic assays

Adult worms of *H. contortus* from stomachs or goats or naturally infected sheep from Ouagadougou slaughterhouse have been used for biological tests (method described by Ademola and Eloff) [16].

Briefly, the stomachs were incised longitudinally by using scissors to release the adult worms. These were carefully collected and placed in a Petri dish containing a solution of phosphate buffer saline (PBS, pH 7.2). Worm's perennials were selected, feces were cleaned by PBS and immediately used for biological tests.

#### 2.3.1. Adult worms motility assay

The adult worms were distributed in Petri dishes (3 worms per Petri dish) in the presence of increasing concentrations (0.1, 1, 3, 10 and 15 mg/mL in PBS) of the plant extract. Levamisole was used as the positive reference substance (1% w:v) and PBS (3 mL) was used as negative control. The assay of the effect of the extract and reference substances on adult worms was performed at 37 °C for 24 h during which motility and survival worms were observed after 2 h, 4 h, 6 h and 24 h incubation.

However, 6 h after incubation, the adult worms in the presence of the different concentrations of the extract and levamisole were returned to the PBS solution for 30 min to observe the possible resumption of motility.

The number of dead worms versus time was evaluated. The test was performed in triplicate and repeated five times.

The mortality rate for each concentration of the extract was determined using the ratio:

$$\frac{\text{Number of dead worms in wells}}{\text{Number of living adults worms in wells}} \times 100$$

#### 2.3.2. Egg hatch assay

The technique described by Ademola and Eloff [16] was used. Briefly, adult worms were collected and washed in PBS (pH 7.2). They were then lightly crushed in a mortar to release the eggs and the resulting mixture was filtered using two mesh sieve (1 mm and 100 µm, respectively). A third sieve of 38 µm was used to retain the released eggs. The sieve was then returned, the opposite side has been washed with distilled water and the eggs were collected in a Petri dish and then distributed at a rate of 1000 eggs/mL.

The *in vitro* anthelmintic activity of the plant extract on the egg hatching of *H. contortus* was carried out according to a modification of the method described by Ademola and Eloff [17]. After extracted from faeces, eggs suspension (100 µL) was distributed in a 24-flat-bottomed microplate so that each well contained 1000 fresh eggs and mixed with 1900 µL of lyophilized extract of *S. senegalensis* at final concentrations of 0.1, 1, 3, 10 and 15 mg/mL. In addition, a negative control (PBS, pH 7.2) and a positive control (levamisole at 1% w:v in PBS) were also included in the assay.

After 48 h of incubation at 25 °C, eggs hatching were stopped by adding formaldehyde in each well. The number of dead or living larvae and eggs per well was then counted under a microscope (Olympus BH-2 Optical Co. Ltd, Japan) at 40× magnification. There were three replicates for control and each concentration which were then repeated five times. The percentage of hatched eggs was calculated using the ratio:

$$\frac{\text{Total final number of eggs in well}}{\text{Total initial number of eggs in well}} \times 100$$

### 2.4. Statistical analysis

The mean percentages of mortality and inhibition of hatching were subjected to analysis with the Prism 5.0 software. The different figures were drawn and LC<sub>50</sub> and upper confidence limits and lower reliability were determined using the same Prism 5.0 software. The results of the pharmacological study were expressed as mean ± SEM. Changes were considered as significant when the probability of error (P) was less than 0.05 (P < 0.05).

## 3. Results

### 3.1. Adult worms motility assay

The anthelmintic activity of *S. senegalensis* and levamisole was increased with incubation time and concentration of the extract (Tables 1 and 2). All the concentrations of *S. senegalensis* showed inhibitory effect on the survival of *H. contortus* in a dose dependent manner. The results showed that the extract of *S. senegalensis* caused mortality of adult worms in a dose dependent manner. Concentrations between 0.10 and 3.00 mg/mL had no effect on motility of worms up to

**Table 1**

Evolution of the percentage of mortality of adult worms *H. contortus* after 4, 6 and 24 h of contact with different concentrations of aqueous decoction of the leaves of *S. senegalensis*.

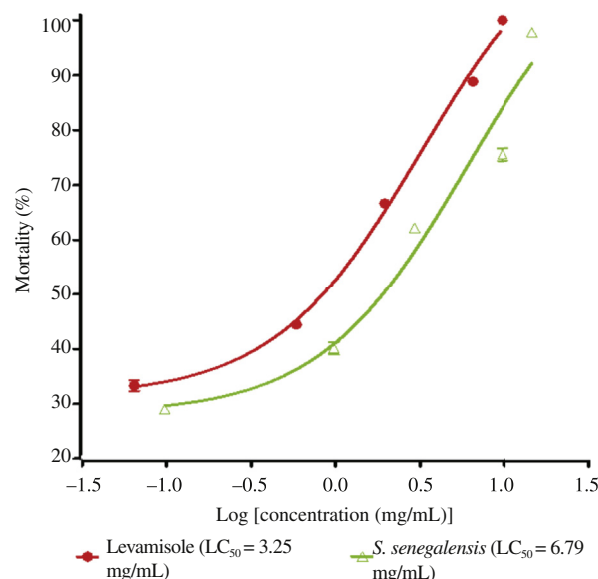
Products	Concentration (mg/mL)	Mortality (% ± SEM, n = 5)		
		4 h	6 h	24 h
PBS	0.00	0.00 ± 0.00	0.00 ± 0.00	14.81 ± 0.57
Aqueous decoction of the leaves of <i>S. senegalensis</i>	0.10	0.00 ± 0.00	2.22 ± 0.44	29.00 ± 0.54
	1.00	0.00 ± 0.00	0.00 ± 0.00	40.11 ± 1.14
	3.00	0.00 ± 0.00	2.22 ± 0.44	62.22 ± 0.54
	10.00	2.22 ± 0.44	11.11 ± 1.22	75.55 ± 1.14
	15.00	2.22 ± 0.44	26.66 ± 0.89	97.77 ± 0.54

**Table 2**

Evolution of the percentage of mortality of adult worms *H. contortus* after 4, 6 and 24 h of contact with different concentrations of levamisole.

Products	Concentration (mg/mL)	Mortality (% ± SEM, n = 5)		
		4 h	6 h	24 h
PBS	0.00	0.00 ± 0.00	0.00 ± 0.00	14.81 ± 0.57
Levamisole	0.07	0.00 ± 0.00	0.00 ± 0.00	33.33 ± 1.00
	0.60	0.00 ± 0.00	11.11 ± 0.57	44.44 ± 0.57
	2.00	0.00 ± 0.00	22.22 ± 0.57	66.66 ± 0.57
	6.66	11.11 ± 0.57	33.33 ± 1.00	88.88 ± 0.00
	10.00	66.66 ± 1.00	77.77 ± 0.57	100.00 ± 0.57

4 h of exposure while the concentrations of 10.00 and 15.00 mg/mL caused a similar mortality (2.22%) on them (Table 1 and Figure 1). It should be noted that the treatment of *S. senegalensis* extract induced a rigid posture or completely immobilized adult worms beyond 4 h. While the minimum concentration of 0.10 mg/mL killed 29.00% of the adult worms after 24 h, a concentration of 15.00 mg/mL killed 97.77% of the worms by the end of the experiment. However, up to 2 h of incubation in the presence of the extract of *S. senegalensis*, no effect on motility or mortality of *H. contortus* adult parasites was shown.



**Figure 1.** Graph showing the effects of aqueous decoction of the leaves of *S. senegalensis* and levamisole on the mortality of adult worms of *H. contortus* in 24 h.

Levamisole induced mortality among treatment according to the time and concentration-dependent with 100% mortality of adults parasites at 10.00 mg/mL (Table 2 and Figure 1).

PBS used as a negative control only induced slight mortality to the order of 14.81% after 24 h of incubation. The concentrations causing the death of 50% (LC<sub>50</sub>) worms were 6.79 mg/mL and 3.25 mg/mL for *S. senegalensis* and levamisole, respectively (Figure 1).

### 3.2. Egg hatch assay

The results of the percentage of the eggs hatch inhibition of *H. contortus* based on different concentrations of *S. senegalensis* and levamisole are shown in Tables 3 and 4. The inhibition of hatching in the presence of *S. senegalensis* extract was in a

**Table 3**

Effects of aqueous decoction of the leaves of *S. senegalensis* at different concentrations on the eggs hatching of *H. contortus*.

Products	Concentration (mg/mL)	Percentage inhibition of hatching (n = 5)
PBS	0.00	18.10 ± 1.16
Aqueous decoction of the leaves of <i>S. senegalensis</i>	0.10	31.81 ± 1.19
	1.00	39.09 ± 0.89
	3.00	51.81 ± 0.89
	10.00	70.90 ± 0.54
	15.00	93.63 ± 0.57

**Table 4**  
Inhibitory effects of levamisole on the egg hatching of *H. contortus*.

Products	Concentration (mg/mL)	Percentage inhibition of hatching (n = 5)
PBS	0.00	18.10 ± 0.89
Levamisole	0.07	22.72 ± 0.57
	0.60	22.72 ± 1.16
	2.00	12.72 ± 0.54
	6.66	12.72 ± 0.54
	10.00	0.00 ± 0.57

concentration-dependent manner. The concentration of 0.10 mg/mL of the extract induced inhibition of 31.81% while the maximum concentration 15.00 mg/mL caused inhibition of hatching of 93.63%. PBS had blocked the hatching of parasite eggs for 18.10%. However, levamisole caused a maximal inhibition of 22.72% with the low concentration of 0.07 mg/mL while high concentrations were less efficacy with 12.72% and 0.00%, respectively for the concentrations of 6.66 and 10.00 mg/mL.

#### 4. Discussion

The aqueous decoction of *S. senegalensis* exhibited anthelmintic activity on *in vitro* studies against eggs hatching and adult worms of *H. contortus*. The aqueous decoction of leaves of *S. senegalensis* on adult worms of *H. contortus* produced a mortality of 97.77% at a concentration of 15.00 mg/mL and its LC<sub>50</sub> was 6.79 mg/mL. This anthelmintic effect of the plant extract may attribute to its content of tannins since several studies indicated that these chemical compounds possess the capacity to bind free protein present in the tubes for larval nutrition that can lead to death [18]. Other studies indicated that anthelmintic drug may diffuse through the intestinal cells to cause their death [19]. Moreover, tannins are well known to possess antiviral [20], antibacterial and anthelmintic activities [21,22]. However, at the same concentration of 10.00 mg/mL, the dose-effect curve for levamisole reached 100% mortality of adult worms of *H. contortus* while the extract of *S. senegalensis* and the negative control PBS were 97.77% and 14.81% after 24 h incubation. It is evident from these results that levamisole (LC<sub>50</sub> = 3.25 mg/mL) used as a positive control had better activity compared with extract of *S. senegalensis* but with slight difference (Figure 1).

Phytochemical screening of *S. senegalensis* plant extract showed the presence of saponins, triterpene glycoside and steroid which might contribute to the anthelmintic activity [23–25] with an independent or synergistic effect [26,27].

The observation of adult worms after treatment shows a reducing in their motility which could have been prevented by the terpenoid compounds present in the extract [19].

In the current study, aqueous extract of *S. senegalensis* possessed an inhibitory effect on eggs hatching of *H. contortus* (Table 3). Indeed, the extract exhibited inhibition 31.81 ± 1.19 and 93.63 ± 0.57 of eggs hatchability at 0.10 mg/mL and 15.00 mg/mL, respectively.

In the PBS solution, 18.10% of the eggs did not hatch. However, eggs hatching need to undergo maturation and therefore require an optimum temperature (20–30 °C) and non-hatching may be due to poor eggs. The average value of inhibition of eggs hatching obtained at 10.00 mg/mL of levamisole is 12.72% while no larva was observed in the solution. This

value contrasts with the properties of this drug which is recognized as an anthelmintic reference. This suggests that levamisole has no inhibitory effect on *H. contortus* eggs or it induces a lysis of eggs by diffusion through the shell egg, hence the decrease in the number recorded based on concentrations. Thus, egg hatch activity of *S. senegalensis* or levamisole may be explained by its possible capacity directly binding to the lipoproteins of the eggshell membrane which induces better permeability leading to their hatching. Likewise, this activity could be due to the binding of plant extract on egg hatching enzymes which raises the rate of hatching [19,28,29].

In the literature, few previous studies have been done on the anthelmintic activity of *S. senegalensis*. Indeed, an ethnobotanical survey conducted in the Ferkessedougou region (Northern Côte d'Ivoire) showed that *S. senegalensis* was one of the most used in traditional veterinary medicine and demonstrated its activity against nematodes [7]. Further results showed that the roots, leaves and fruits of *Tabernaemontana citrifolia*, species belonging to the same family as *S. senegalensis* possess similar results on eggs and adult worms of *H. contortus* [30].

The present *in vitro* study of the effects of aqueous decoction of leaves of *S. senegalensis* showed anthelmintic activity on adult worms and possessed an inhibitory effect on eggs hatching of *H. contortus*. Taking together, *S. senegalensis* would have anthelmintic properties that may justify its use in traditional medicine to treat gastrointestinal parasites.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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