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Ovicidal and Larvicidal Activities of *Saba senegalensis* (A.DC) Pichon (Apocynaceae) Extracts and Fractions on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MBB carried out experimentation and article writing. Author AT evaluate the tests and correct version of the article. Author LB for data analysis. Author FBK to supervise the phytochemical study. Author SO to supervision the pharmacological tests and author IPG for the general supervision of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate ovicidal and larvicidal activities of an aqueous decoction (AD) and hydroethanolic macerate (HEM) extracts and fractions of the leaves of *Saba senegalensis*.
Study Design: *In vitro*, the ovicidal and larvicidal activities of AD and HEM extracts and fractions of the leaves of *Saba senegalensis* on the eggs and larvae (L1) of *Heligmosomoides bakeri*.
Place and Duration of Study: The experiment was conducted at the department of Medicine and Traditional Pharmacopeia-Pharmacy (MEPHATRA-PH) of Institute of Research in Health Sciences (IRSS) between June 2015 and December 2016.

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Methodology: The phytochemical groups of the extract and fractions of *Saba senegalensis* were determined by a colorimetric and Thin Layer Chromatography methods. The eggs were obtained from feces of mice deliberately infected and the larvae from the eggs were incubated at 25 ±2℃ for 72 hours. Eggs and larvae were exposed to increasing concentrations (100; 625; 1250; 2500; 3750 µg/mL) of the different extracts, 48 hours and 24 hours for the eggs and larvae respectively. Distilled water and DMSO 0.1% were used as negative controls while albendazole and levamisole were used as positive controls.
Results: The phytochemical groups of interest are the tannins, saponins, flavonoids and triterpenes. The negative control had given 2.16% of egg hatch inhibition and 0% of larvae mortality mean while the positive control had given 100% in both cases. The extracts inhibited eggs hatching and affected larval survival. Pharmacological effects were concentration-dependent. The ovicidal and larvicidal activity of HEM is more interesting than that of AD with an Emax = 95.60% and an IC_{50} = 390 µg/mL. It is the same for the larvicidal activity with Emax = 100% and an LC_{50} = 900 µg/mL. However, the differences were not statistically significant.
Conclusion: These results show the ovicidal and larvicidal properties of the *S. senegalensis* leaves.

Keywords: Saba senegalensis; Anthelmintic; Heligmosomoides bakeri; in vitro; Burkina Faso.

1. INTRODUCTION

Neglected tropical diseases affect more than a billion people in 149 countries, the majority of whom live in warm climate regions and constitute a public health problem. [1]. These diseases cause enormous suffering and increase poverty in the concerned populations indicating a real health constraint. In parasitised hosts, the presence of worms is most often known by causing gastrointestinal disorders (diarrhoea, stomach pains), respiratory disorders (cough), etc. In most cases, symptoms of parasitic infections are accompanied by severe anemia and vitamin A deficiency [2]. In tropical regions, helminthiasis is the most prevalent parasitic infections and cause significant economic losses [3]. Thus, control measures are needed through the use of modern medicines. Moreover, in some situations, the use of anthelmintic drugs has led to the development of resistance to available molecules [4]. Thus, it becomes urgent to find and promote innovation for the discovery and development of new antiparasitic drug with better efficacy, efficiency and tolerability [5]. Indeed, according to the World Health Organization (WHO, 2002), more than 80 % of the population uses traditional medicine for their needs in primary health care [6]. A great interest for the discovery of new antiparasitic drugs from medicinal plants extracts were undertaken in numerous research centres and laboratories worldwide. Studies on *Carica papaya* and *Duranta erecta* antiparasitic plants found in the tropical region of Africa, extract have shown anthelmintic properties *in vivo* through mice infection model and also against the eggs and larvae of *Heligmosomoides bakeri* (*H. bakeri*) [7, 8]. Others medicinal plants were evaluate for their anthelmintic potential activities in Australia [9] and Brazil [10].

In Burkina Faso on the anthelmintic effect of *Acacia nilotica* and *Acacia raddiana* [11] were demonstrated. Moreover, a combination of *Cassia sieberiana*, *Guiera senegalensis*, *Sapium grahamii* three plants used in traditional medicine also showed anthelmintic effects on adult worms and eggs of *Haemonchus contortus* (*H. contortus*) [12]. This study was undertaken to contribute to the valorisation of *Saba senegalensis* (*S. senegalensis*) which is widely used in traditional medicine in Burkina Faso against parasitic. Indeed, an ethnobotanical survey conducted by researchers from the Research Institute for Health Sciences (IRSS) have shown that the leaves of this plant were used for the treatment of parasitosis [13] and the anthelmintic effect of this part of *S. senegalensis* on adult worms and eggs of *H. contortus* has also been shown [14]. This study aims to investigate *in vitro*, the ovicidal and larvicidal activities of an aqueous decoction and hydro-ethanolic fractions of the leaves of *S. senegalensis* on the eggs and larvae (L_1) of *H. bakeri.*

2. MATERIALS AND METHODS

2.1 Plant Collection

Leaves of *S. senegalensis* (A.DC) Pichon were collected around Bassinko, a department located about 30 km at the north of Ouagadougou (Savana area), in July 2015. A sample of the plant was identified by plant taxonomist at the

herbarium of the National Centre for Scientific and Technological Research (CNRST) and a voucher specimen was deposited under No. 00223 HNBU. The plant's leaves were air dried at room temperature, powdered using pestle and mortar and kept in amber colored bottles until use in order to keep all their physicochemical properties.

2.2 Extracts Preparation

The extractions were carried out at the chemical laboratory of the Medicine Pharmacopoeia Traditional and Pharmacy (MEPHATRA/Ph) department at the IRSS.

2.2.1 Preparation of hydroethanolic macerate

A solid-liquid extraction method was carried out at room temperature. It is usually used for the extraction of heat sensitive compounds. The aim of this maceration is to extract soluble substances in alcohol. For this purpose, a sample (50 g) of the powder is macerated for 24 hours in 1 L of ethanol-Water (v/v at 70/30). After filtration followed by a centrifugation (2000 rev/min for 5 minutes) the filtrate is oven dried at rotavapor, hydroethanolic macerate (HEM) was used for the different experiment.

2.2.2 Preparation of aqueous decoction

A decoction of *S. senegalensis* (A.DC) Pichon was prepared by soaking 50 g of the dry powder in distilled water (500 mL) and the mixture was boiled for 45 min. After cooling, the decoction obtained was filtered through a whatman paper with nylon cloth and then centrifuged at 2000 rpm for 5 min. The supernatant was collected and a portion was concentrated in an oven at 50°C for 24 h, frozen and then lyophilized. The aqueous decoction (AD) lyophilized dry powder was then collected in a stoppered sample vial, weighed and kept in a desiccator to avoid water absorption until use for an assay.

2.2.3 Fractionation of *S. senegalensis* extracts

The principle is based on the degree of solubility of the chemical groups in organic solvents.

2.2.3.1 From hydroethanolic macerate

Extraction was performed on 200 g of the raw material mixed with 1 L of ethanol in a beaker before being introduced into a glass column (2 L). The drug used is previously moistened with ethanol before introduction into the column. After 24 hours of maceration, lixiviation is carried out and then the collected extract is subjected to evaporation at low pressure at 35°C. Lixiviation is an extraction process which consists in leaching products with a specific solvent to extract the soluble parts of the product. A concentrated portion of the extract obtained following extract with dichloromethane was retained (F_{HEM}-DCM) and the other portion was used for liquid-liquid separation with ethyl acetate. At the end of the separation, the extract with ethyl acetate (F_{HEM}-AcOEt) collected is subjected to evaporation at low pressure at 35°C and the residual aqueous phase (F_{HEM}-Residue) is kept. The F_{HEM}-DCM and F_{HEM}-AcOEt fractions in one part and the residue in the other part were evaporated to dryness and then preserved for phytochemistry and pharmacological assays.

2.2.3.2 From aqueous decoction

The extraction is carried out on 400 g of the raw material mixed with 2 L of distilled water in an extraction flask. After the decoction, the supernatant was collected and dried a heat chamber at 50°C for 24 h. The extract collected is subjected to evaporation at low pressure at 35°C before fractionation. A concentrated portion of the extract obtained following leaching with dichloromethane is retained (F_{AD}-DCM) and the other portion is used for liquid-liquid separation with ethyl acetate. At the end of the separation, the extract with ethyl acetate (F_{AD}-AcOEt) collected is subjected to evaporation at low pressure at 35°C and the residual aqueous phase (residue) is preserved.

The F_{AD}-DCM, F_{AD}-AcOEt and F_{AD}-Residue fractions are evaporated to dryness and stored for phytochemical and pharmacological assays.

2.3 Phytochemical Screening of Extracts

Aqueous decoction and hydroethanolic macerates were used in order to determine the different chemical groups present in the extract tested. It was carried out by chemical characterizations in a liquid medium reaction [15].

2.3.1 Characterization in liquid medium and with acid hydrolysate

The characterization in liquid medium consisted to highlight the unhydrolyzed aqueous extract and hydrolyzed chemical groups of pharmacological interests. The chemical groups sought were: phenolic compounds (Reaction with

$FeCl_3$), saponosids (test of foam index), reducing compounds (Reaction of Fehling), oses and polyoses (reaction with resorcinol and iodine), salt alkaloids (Reaction of Dragendorf). The characterization with acid hydrolysate was carried out to highlight flavonoids (reaction of shibata), steroidal and triterpenic glucosides (Reaction of Liebermann-Buchard), antracenosides (reaction of Bornträger), coumarins and derivatives (reaction of Feigl) [15].

2.3.2 Characterization using Thin Layer Chromatography

The phytochemical screening was performed on silica gel chromatoplates 60 F254 (Merck, Germany) according to the methods described by Lhuilier (2007) [16]. Different solvent systems were used: Ethyl acetate / Methanol / H_2O (7/2/1) for AD, F_{AD}-Residue, HEM, F_{HME}-Residue as well as for the acetate Ethyl acetate and the Toluene / ethyl acetate / glacial acetic acid (5/4/1) system for the dichloromethane fractions. According to the type of secondary metabolite to be identified, several specific reagents have been used. It was a solution of 1% $FeCl_3$ in 80% ethanol for tannins, a solution of sulfuric anissaldehyde for saponins, NEU reagents for flavonoids and a solution of sulfuric vanillin or with Libermann Buchard reagents for sterols and triterpenes.

2.4 Anthelmintic Assays

2.4.1 Recovery of nematode eggs

Fresh eggs of *H. bakeri* were obtained from the feces of previously experimentally infected mice according to Ngangout et al. (2012) [17]. Briefly; 03 g of feces were collected, homogenised in a motar, suspended in saturated salt solution and cleaned of organic debris by filtration through sieves (1 000 µm and 150 µm) into a 100 mL beaker. The content of the latter was poured into four test tubes until the formation of the meniscus. A cover slides was deposited on each tube; after about 5 min, they were collected and deposited on slides and then examined using a microscope at 4X magnification for the confirmation of the presence of eggs. Slides and cover slide containing eggs were rinsed with distilled water into a beaker (100 mL). Eighty (80) milliliters of distilled water were added into the beaker and allowed to stand for about 01 hour to clean the eggs of salt solution. The beaker was left to stand for one hour for sedimentation of the eggs. The supernatant was carefully removed using a syringe. The suspension of the eggs was then concentrated between 30 and 40 eggs / mL in a conical tube for testing the ovicidal activities.

2.4.2 Evaluation of the ovicidal activity

The method used was previously described [17]. To assess the effects of extracts on fresh eggs, 1 mL of a suspension of 30-40 eggs/mL was dispensed into Petri dishes. One (01) mL of extract was added in each dish so as to obtain increasing concentrations of 100; 625; 1250; 2500; 3750 µg/mL. Albendazole was used as a positive control at increasing concentrations (1; 6.25; 12.5; 25; 37.5 µg/mL) and the distilled water was used as the negative control. Petri dishes were covered and the eggs incubated at room temperature (25 °C). After 48 h, the number of eggs and larvae L_1 per plate was counted using a microscope (x 10). The hatching percentage (E%) was determined using the following formula.

$$E\% = \frac{\text{Number of L1 larve}}{\text{Number of eggs in culture}} \times 100$$

2.4.3 Recovery of nematode larvae

The eggs were cultured according to the technique described by Wabo (2013) [18]. Indeed, 0.5 g of feces was cultured on whatman paper in Petri dishes in the presence of activated carbon. The dishes were kept in a moist environment for 7 days (Temperature 4°C). After one week, the larvae were recovered by rinsing the edges of the filter paper. The egg suspension was pipette into 10 mL tubes and then centrifuged at 2000 rpm for 5 min. After centrifugation, the supernatant was carefully siphoned using a pipette and the bottom containing a concentrate of L_1 larvae was stored in a flask containing 5 mL of Ringer solution to ensure their survival and kept at 4°C in the refrigerator until use.

2.4.4 Evaluation of larvicidal activity

The protocol was already described by Wabo (2013) [18]. To assess the effects of the extracts on L_1 and L_2 larvae, 1 mL of a solution containing about 30-40 larvae was distributed in each of the 18 Petri dishes (35 mm Ø x 10 mm) and mixed with the same volume of a specific extract giving the following final tested concentrations (100; 625; 1250; 2500; 3750 µg/mL). All the tests were repeated 6 times for each treatment and control. Levamisole (0.1%) was used as a positive control (concentrations 1; 6.25; 12.5; 25; 37.5

µg/mL) and the distilled water as a negative control. The Petri dishes were covered and kept at room temperature for 24 h, after which the number of dead or immobile larvae was counted under a microscope (at 4 X magnification).The percentage of mortality (Mc%) was determined to use Abott's formula [17]:

$$M_c\% = \frac{M_{ce} - M_t}{100 - M_t} \times 100$$

Where:

Mc (%) represents the corrected mortality,
M_{ce} represent the mortality obtained during the test,
M_t is the mortality registered in the negative control dishes.
When the mortality rate in the latter dishes is less than 5%, $M_c = M_{ce}$.

2.5 Statistical Analysis

Results were expressed as mean ± ESM. The results analysis of the *in vitro* tests was carried out on the basis of statistical processing using Graph Pad Prism software version 5.0. The comparison of the different groups was carried out using One way-ANOVA, followed by the Dunett multiple comparison test (treated groups vs control). The differences were considered to be statistically significant at $p < 0.05$ compared to the control. The lethal and inhibitory concentrations 50 per cent (IC_{50} and LC_{50}) were determined using the regressive line of the probit according to the decimal logarithm of the concentration. All tests were repeated six times (n=6) in triplicate for each treatment and control.

3. RESULTS

3.1 Phytochemistry

3.1.1 Characterization in liquid medium and with acid hydrolysate

The results of the phytochemical screening of the aqueous decoction and hydroethanolic macerate of *S. senegalensis* are shown in the Table 1.

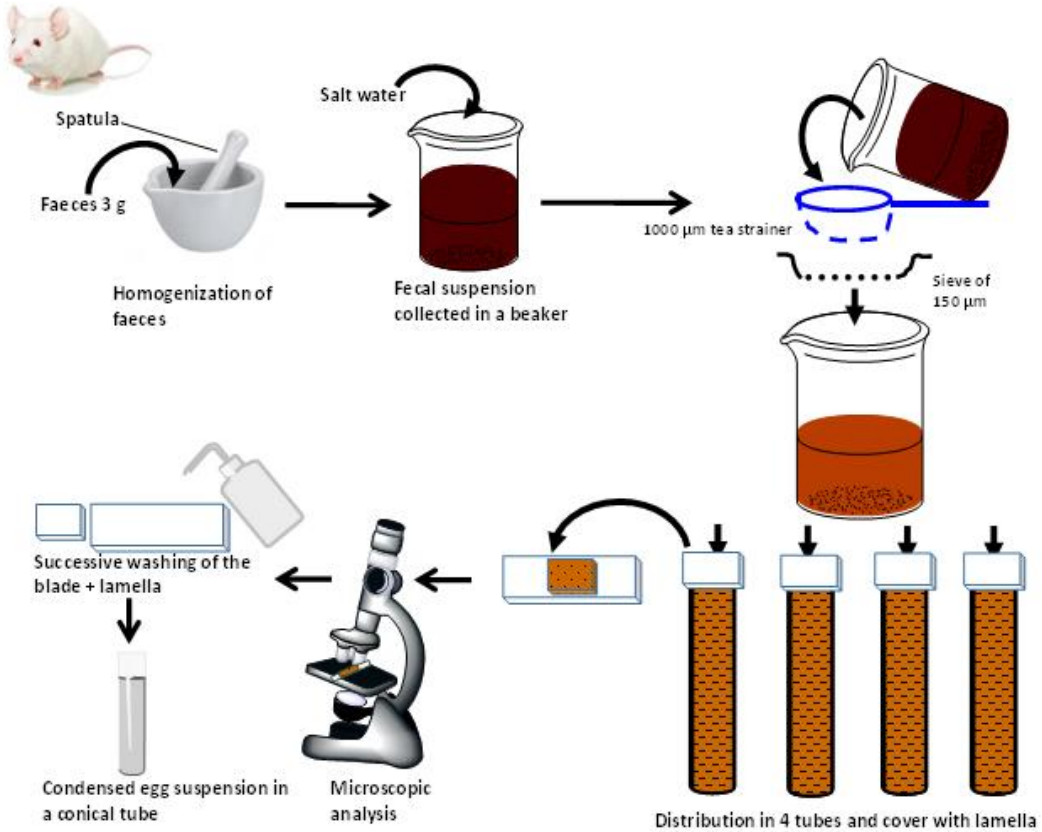

Fig. 1. Procedure for the recovery of *H. bakeri* eggs

Table 1. Phychemical groups highlighted in the aqueous decoction and hydro-ethanolique macerate of the leaves of *Saba senegalensis* (A.DC) Pichon

Chemical group	Extracts	
	Aqueous decoction	Hydroethanolic macerate
Alkaloids salts	nd	-
Tannins	+	+
Saponins	+	+
Reducing compounds	+	+
Oses	+	+
Polyoses	-	+
Anthocyanosides	+	+
Flavonoids	+	+
Steroid and triterpenic glucosides	+	+
Antracenosides	-	-
Coumarins and derivatives	+	+

(+) indicates presence, (-) indicates absence and (nd) indicate not determined of that chemical constituent in the plant sample

The results show an abundant presence of tannins, flavonoids, saponins, reducing compounds, oses, anthocyanosids, steroïdic and triterpenic glucosides, coumarins and derivatives in these two extracts. The chemical screening did not reveal the presence of polyoses, flavonoids and antracenosides in the aqueous decoction and hydroethanolic macerate of *S. senegalensis*. Also, the presence of alkaloids salts did not reveal in the hydroethanolic macerate.

3.1.2 Characterization using thin layer chromatography

The TLC plates showing the presence of different secondary metabolites that are shown in the Fig. 2.

The results confirmed the presence of tannins, sterols and triterpenes, flavonoids, saponins in the extract of *S. senegalensis*.

3.2 Anthelmintic Assay

3.2.1 *In vitro* assay of aqueous decoction and fractions of *S. senegalensis* on the eggs of *Heligmosomoides bakeri*

The study showed that the leaves of *S. senegalensis* have an ovicidal activity on the eggs of *H. bakeri* as illustrated in the following graphs.

3.2.1.1 Effect of aqueous decoction and fractions on eggs of H. bakeri

The Fig. 3 show IC_{50} of the ovicidal effect of the aqueous decoction of *S. senegalensis* and its fractions on eggs of *H. bakeri*.

The IC_{50} values were of 631.23 ± 41.57, 1274.1 ± 156.35, 1156.8 ± 353.17, 807.76 ± 5.23 µg/mL for the AD, F_{AD}-DCM, F_{AD}-AcOEt and F_{AD}-residue respectively. These results showed that the aqueous decoction of *S. senegalensis* and the residual fraction (F_{AD}-residue) are more powerful on the eggs of *H. bakeri* than those of the F_{AD}-DCM and the F_{AD}-AcOEt fractions. However, the best activity was obtained with the positive reference (Albendazole, IC_{50} = 1.47 ± 0.36 µg/mL).

3.2.1.2 Effect of hydroethanolic macerate and fractions on eggs of H. bakeri

The Fig. 4 showed an IC_{50} histogram of the ovicidal effect of the hydroethanolic macerate of *S. senegalensis* and its fractions on eggs of *H. bakeri*.

The analyze of Fig. 4 showed that the hydroethanolic macerate of *S. senegalensis* presents an interesting activity on eggs compared to fractions (HEM, F_{HEM}-DCM, F_{HEM}-AcOEt and F_{HEM}-Residue). The IC_{50} values were 401.03 ± 60.87, 999.8 ± 61.00, 1060.5 ± 39.25 and 694.9 ± 81.45 µg/mL for the HEM, F_{HEM}-DCM, F_{HEM}-AcOEt and F_{HEM}-Residue respectively. Difference between HEM and the fractions but this activity is low compared to those of Albendazole (IC_{50} = 1.47 ± 0.36 µg/mL).

3.2.2 *In vitro* assays of extracts and fractions of *S. senegalensis* on L_1 larvae of *H. bakeri*

The study showed that the leaves of *S. senegalensis* have larvicidal activity on L_1 larvae of *H. bakeri* as shown in the following graphs.

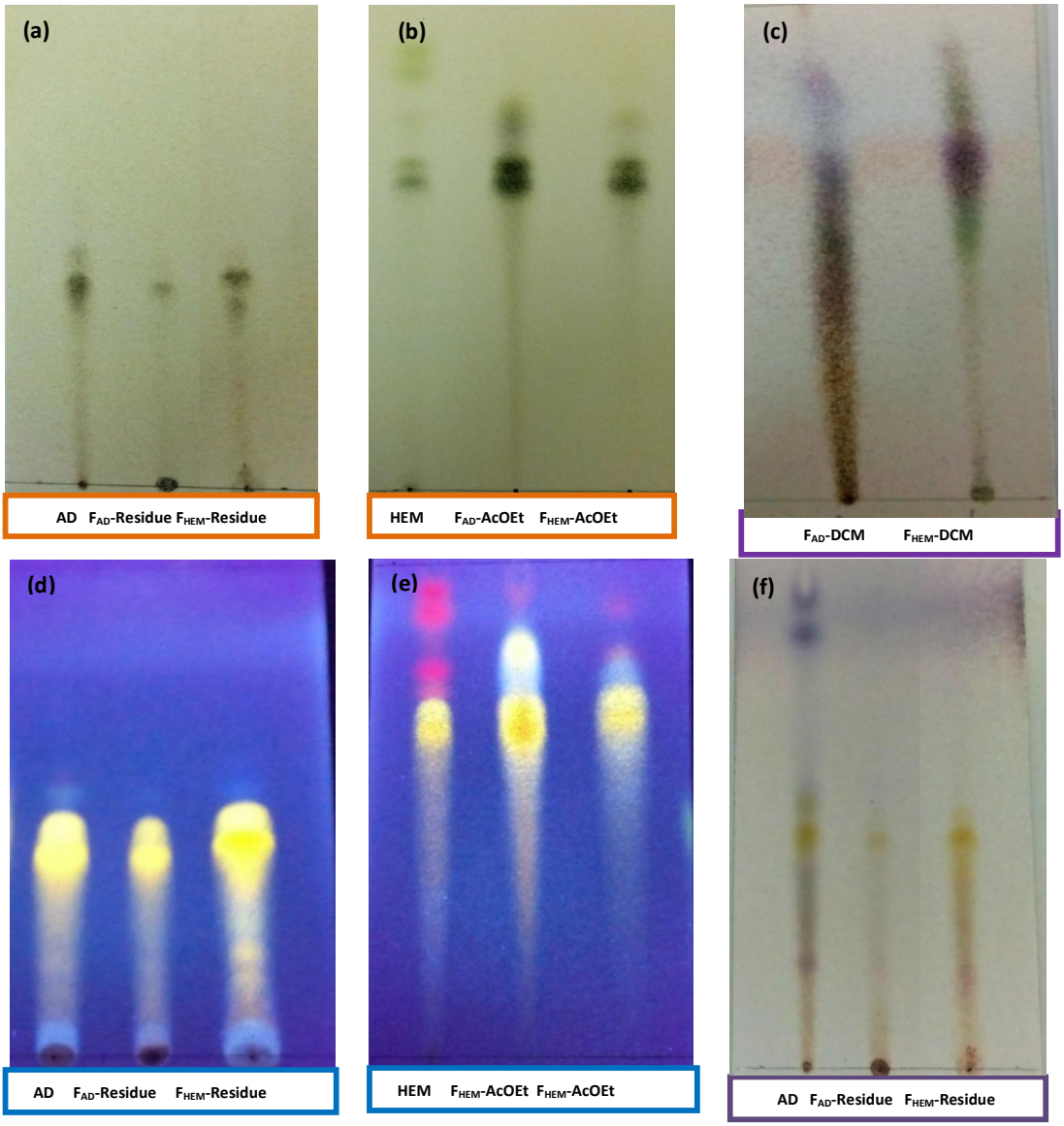

Fig. 2. Chromatographic profile showing the presence of tannins, sterols and triterpenes, flavonoids and saponins. (a) Tannins (at visible); (b) Tannins (at visible); (c) Sterols and *triterpenes (at visible); (d) Flavonoids (at 366 nm); (e) Flavonoids (at 366 nm); (f) Saponins (at visible)*

AD=Aqueous decoction Fraction; F_{AD}-DCM=Dichloromethane of Aqueous decoction; F_{AD}-AcOEt=Fraction Ethyl acetate of Aqueous decoction; F_{AD}-Residue=Residual fraction of Aqueous decoction; HEM=Hydroethanolic macerate; F_{HEM}-DCM=Fraction Dichloromethane of Hydroethanolic macerate; F_{HEM}-AcOEt=Fraction Ethyl acetate of Hydroethanolic macerate; F_{HEM}-Residue=Residual fraction of Hydroethanolic macerate

3.2.2.1 Effect of aqueous decoction and fractions on larvae of H. bakeri

The Fig. 5 showed an LC_{50} of the larvicidal effect of the aqueous decoction of *S. senegalensis* and its fractions on larvae of *H. bakeri*.

The LC_{50} values were of 1034.83 ± 229.39, 712.86 ± 26.96, 935.56 ± 191.71, 727.06 ± 37.30 µg/mL for the AD, F_{AD}-DCM, F_{AD}-AcOEt and F_{AD}-Residue respectively. These results demonstrated that the F_{AD}-DCM of *S. senegalensis* and the F_{AD}-Residue are more powerful on the eggs of *H. bakeri* than those of the AD and the F_{AD}-AcOEt fractions. The best activity was obtained with the positive reference (Levamisole, LC_{50} = 61.46 ± 37.30 µg/mL).

3.2.2.2 Effect of hydroethanolic macerate and fractions on larvae of H. bakeri

The Fig. 6 showed an LC_{50} histogram of the larvicidal effect of the hydroethanolic macerate of *S. senegalensis* and its fractions on larvae of *H. bakeri*.

The analyse of Fig. 6 show that the hydroethanolic macerate of *S. senegalensis* presented an interesting activity on eggs compared to fractions (HEM, F_{HEM}-DCM, F_{HEM}-AcOEt and F_{HEM}-Residue). The LC_{50} values were 964.53 ± 47.75, 1015.46 ± 61.92, 403.13 ± 74.54 and 619.23 ± 56.76 µg/mL for the HEM, F_{HEM}-DCM, F_{HEM}-AcOEt and F_{HEM}-Residue respectively. Fig 6 indicate a difference between HEM and the fractions but this activity is low compared to those of Levamisole (LC_{50} = 61.46 ± 37.30 µg/mL).

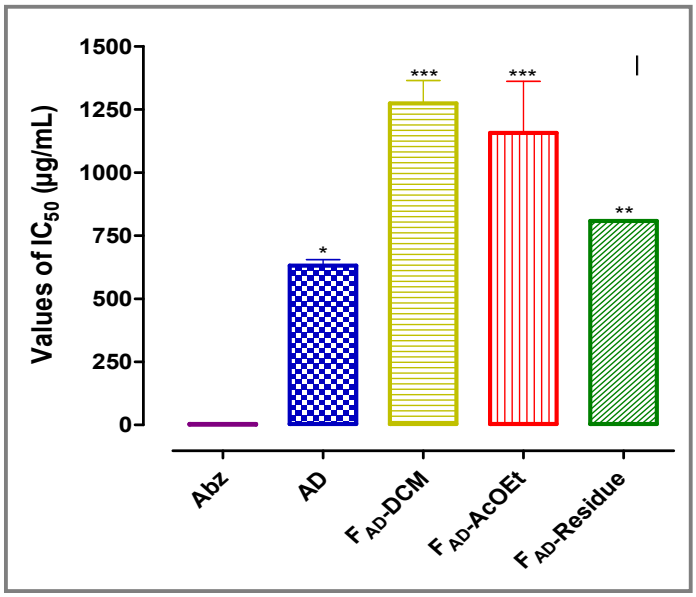

Fig. 3. IC_{50} of ovicidal effect (n = 6; **p <0.05 versus Abz)*

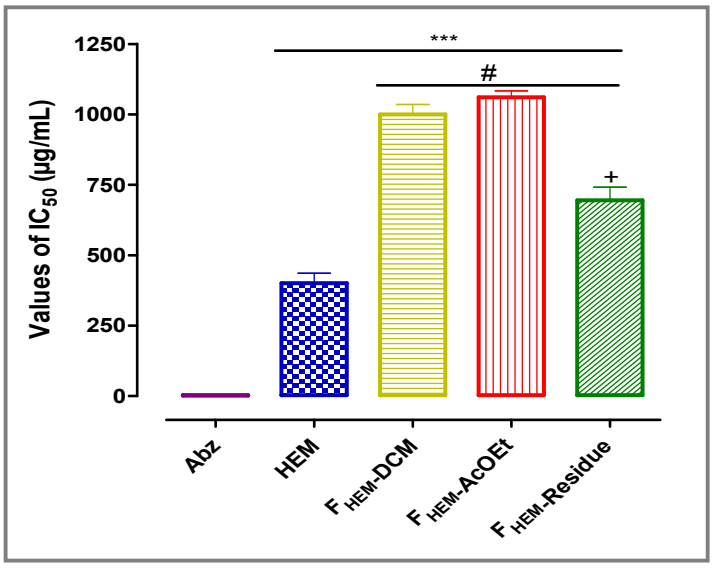

Fig. 4. IC_{50} of ovicidal effect

*(n = 6; *p<0.05 versus Abz; #p<0.05 versus HME and +p<0.05 versus F_{HME}-DCM)*

4. DISCUSSION

The *in vitro* assays tests were used in this study. These tests promoted the determination of anthelmintic activity of drug directly on the eggs or on the process of development of parasites [17,19]. The main advantages of this method for antiparasitic properties of medicinal plant extracts detection were the simplicity of the protocol, the low cost and the speed for large-scale screening. The phytochemical study of the AD and HEM of the leaves of *S. senegalensis* (A.DC.) Pichon by the characterization tests revealed a variety of chemical compounds that are tannins, flavonoids, saponins, reducing compounds, oses, anthocyanosides, steroidal and triterpenic glucosides, coumarins and derivatives (Table 1). The presence of all these compounds were confirmed by the TLC plate (Fig 2). These results are in agreement with those of Nacoulma, (1996) [20] who raised the presence of tannins, flavonoids, saponins, reducing compounds, oses, anthocyanosides, steroidal and triterpenic glucosides in the of *S. senegalensis* plant. Other authors raised the presence of carotenoids, anthraquinones, sterols and triterpenes in the fruits of *S. senegalensis* indicating the wealth of chemical compounds of this plant. The diversity of the chemical groups presents in the extracts tested could give them various biological activities. It is the example of the polyphenols compounds that are well known to have anti-inflammatory, vitamin, bacteriostatic, bactericidal, antioxidant, vasodilating [21] and antiparasitic properties [22,23].

The results of the present study showed that the different extracts have an ovicidal effect *in vitro* on eggs of *H. bakeri*. The best ovicidal effect was obtained with the AD and its residual fraction compared to F_{AD}-DCM and F_{AD}-AcOEt but without significant difference. However, these extracts produced a much lower ovicidal effect than Abz (positive control), which caused a 100% inhibition of eggs hatching of *H. bakeri* (Fig. 3).

Similarly, the HEM induced an ovicidal effect on *H. bakeri* eggs significantly different to those of the residual fractions, the F_{HEM}-DCM and the F_{HEM}-AcOEt. The results also showed that the residual fraction of HEM had a better egg hatching inhibitory effect than the F_{HEM}-DCM and F_{HEM}-AcOEt with a significant difference (Fig. 4). When compared AD and HEM for their egg hatching activity, the HEM presents a relative high effect but without significant difference to AD.

The difference on the ovivdal activity of the tested extracts may be due to the nature of solvent and the process of extraction, but also to the proportion of antiparasitic biochemical substances contained in the extracts [18].

In addition to the ovicidal activity of *S. senegalensis* extracts, the present findings showed an interesting larvicidal effect. Indeed, all extracts and fractions induce a death at 100% of *H. bakeri* larvae with the exception to AD (82.70 ± 8.44) and F_{HEM}-Residue (91.56 ± 9.15) (Figs. 5-6).

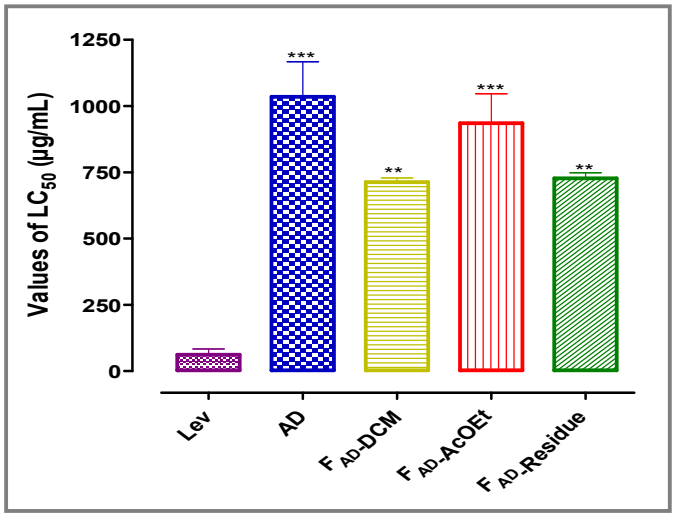

Fig. 5. LC_{50} of larvicidal effect (n = 6; **p<0.05 versus Lev)*

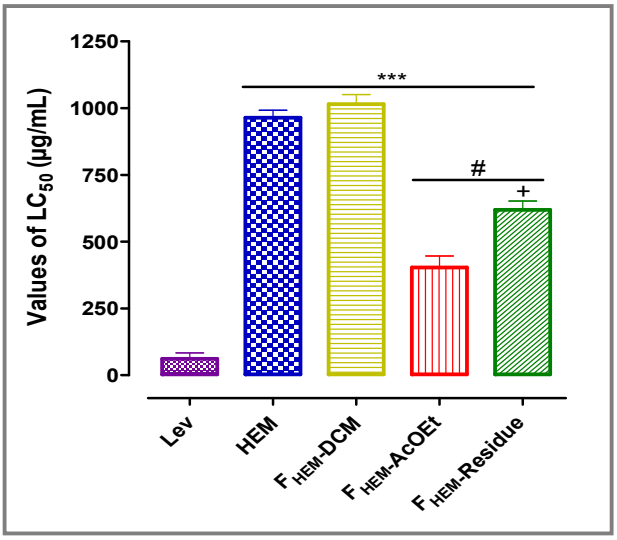

Fig. 6. LC_{50} of Larvicidal effect
*(n = 6; *p<0.05 vs Lev; #p<0.05 vs HME and +p<0.05 vs F_{HME}-AcOEt)*

Statistical analysis of the larvicidal activities showed that the LC_{50} of the AD and the fractions from AD did not show no significant differences between them, while the F_{HEM}-AcOEt showed the best activity compared to HEM and the other fractions from HEM. Therefore, all the tested extracts possess a larvicidal activity relatively lower and significantly different compared to the levamisole used as positive control. Nevertheless, the F_{HEM}-AcOEt presented the most powerful larvicidal effect on L_1 larvae of *H. bakeri* whereas, the AD possesses the weakest effect with LC_{50} values of 403.1 µg/mL and 1034.8 µg/mL respectively. This result may be due to the difference in the process of extraction since studies indicated a best action of apolar compounds such as terpenes and tannins on larvae of nematodes. During this study, the DMSO (at 0.1%) was used as negative control and did not impact on eggs hatching and larvae development (data not show). These results are in line of other authors which indicated that DMSO is tolerated by eggs and larvae of nematodes [18].

The ovicidal and larvicidal effects of the extracts tested in this study could also be explained by a synergistic action of their contained chemical compounds which interact either directly by abrasive effect on the cuticle of larvae or by blocking the cycle of egg evolution. Moreover, the presence of tannins in the plant extracts was interesting and may permit to better understand their action on eggs and L_1 larvae [24,25]. Indeed, these chemical substances can pass through the various layers of the eggs and inhibited the blastomeres mitosis. In this case, the mode of action would be similar to those observed for the compounds of the benzimidazole family [26]. The blocking of the blastomeres is known to be sensitive to the segmentation stage of the eggs which are not fertilised. In the literature, the nematocidal activity of tannins is also well known [23,27]. These molecules are rich in glycoproteins and generally bind to the free proteins or larval cuticles in order to reduce nutrient availability which in turn leads to larval death due to the famine [27]. Moreover, on the eggs and larvae of the parasite, the tannins have the ability to bind to the proteins and change their physical and chemical properties. Thus, by attaching to the cuticle of nematodes rich in hydroxyproline, the tannins cause its rupture [18]. These mechanisms of action may explain the possible effect of the tested extracts of *S. senegalensis*.

In the same case, saponins are very present in the extracts of *S. senegalensis* and may contribute to the death of the L_1 larvae. Indeed, saponins generally interact with cell membrane that cause changes in their structure leading to membrane permeability and damaging action for nematodes. They can also interact with the collagen proteins of the cuticle of nematodes leading to their destruction [28].

The larvicidal activity of the extracts may also be explained by the flavonoids that they contained. Indeed, studies indicate that the presence of

flavonoids in plant extracts may affect moulting and survival of larvae and may improve the activity of other chemicals compounds [29,30]. Moreover, the anthelmintic activity of flavonoids is known since these compounds can inhibit key biological processes like egg hatching, larval development and affect adults' worms in *Caenorhabditis elegans* [31]. Interestingly, the biochemical and pharmacological activities of flavonoids have been attributed to their anti-oxidative and free-radical scavenging properties [32] and *S. senegalensis* extracts are known to have antioxidant activity [33] and may justify the effect of extracts investigated in this study.

5. CONCLUSION

Taken together, the present study revealed that *Saba senegalensis* is a potential anthelmintic. The ovicidal and larvicidal activity of the different extracts could be attributed to the presence of tannins, flavonoids, saponins, reducing compounds, oses, anthocyanosides, steroidal and triterpenic glucosides, coumarins and derivatives in this plant extracts. However, these results underlined the need to further perform a bioguided biochemical analysis of the various fractions to identify precisely which are active compounds even if the synergistic action of the chemical groups did not to be excluded.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The laboratory experimentation was carried out according to the experimental protocols validated by the MEPHATRA-PH laboratories and meeting the international standards in this field (guidelines established by the European Union on the protection of animals, CCE Conseil 86/609). These different experiments were carried out on the mouse and the parasites, *Heligmosomoides bakeri* and did not concern in any case the human subject. These protocols are ethical to experiment on laboratory animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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