

Phytochemical Study and *In vitro* Anthelmintic Properties Studies of the Trunk Barks Aqueous Extract from *Acacia Nilotica* Var. *Adansonii* (Guill & Perr). O Ktze (Mimosaceae)

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ABSTRACT

The present study was to estimate the *in vitro* anthelmintic effect of the aqueous extract of the trunk barks of *Acacia nilotica* var. *adansonii*, plant used in this traditional medicine against gastro-intestinal parasites. Trunk barks of *Acacia nilotica* var. *adansonii* were used as plant material, eggs and adults worms of *Haemonchus contortus* were the animal material used. The adult worms and the eggs were put in contact with increasing concentrations of the extract. A phytochemical screening of the plant material was also performed. This study revealed the presence of chemical groups with anthelmintic properties such as tannins, triterpenics, saponosides in the aqueous extract. The vermicide effect was indicated by the lethal concentration of 50% (LC₅₀) of adult worms equal to 1.28 mg / mL compared to the levamisole LC₅₀ which was 3.25 mg / mL. The rate of eggs hatching inhibition was 93.84 % at the extract concentration of 0.1 mg / mL. Anthelmintic properties of *Acacia nilotica* var. *adansonii* would be real, which justifies its use in traditional medicine for the treatment of gastrointestinal parasites

Keywords: *Acacia nilotica* var. *adansonii*, phytochemistry, anthelmintics, Burkina Faso.

INTRODUCTION

The poor sectors of society of developing countries, in particular those of sub-Saharan Africa always pay a heavy tribute from diseases caused by the parasitic worms of the digestive tract. According to WHO in 2013, a billion people in the world are affected by helminthiasis and about two billions people incur a risk of affections. Helminthiasis constitute a problem of public health in sub-Saharan Africa with about 200.000 deaths each year¹.

In Burkina Faso prevalence of the intestinal parasitoses was 54.7% between 1997 and 2007². A recent study conducted by Sangaré et al³ in Bobo Dioulasso indicated a prevalence of 65.3 % for the infections by gastrointestinal parasites.

These helminthiasis are often causing of obvious morbidity such as the hurts and the intestinal obstructions, the dermic hurts, losses of blood causing anaemia. They are often responsible of nutritional deficiency which can be heavy of consequences for the children and childbearing age and pregnant women⁴. Thus, these diseases undermine the intellectual growth, the cognitive development and the school performance of these children. They also lead to the decrease of efficiency in the work which consequences are a social decline and imbalance⁵.

The sector of the breeding is not outdone with regard to the damage that intestinal parasites cause. The economic losses caused by these parasites in this sector in the world

are very important, especially in regions with agriculture and pastoralism specificity⁶. These losses were considered at approximately 45 million US dollars in South Africa according to Waller⁷. In Burkina Faso they rage in an endemic way with prevalence which varied between 70 to 100 %^{8,9}. With regard to the problem of public health and the socioeconomic impact that they cause, a number of measures and methods of fight were undertaken to overcome these parasitosis.

So, the purification and the sensitization to the practice of the hygiene in everyday life are preventive measures recommended by the OMS¹⁰.

But the control of these parasitosis remains for the moment based on the use of anthelmintic medicines¹¹ even in prevention⁴.

The excessive use of these synthetic anthelmintics generates some negative consequences for the users. Indeed, besides the increase of the unwanted effects, we assist to a development of the resistance of helminthes. In veterinary medicine, it's established chemo resistance at the benzimidazoles and lévamisole¹². The problem of accessibility at the appropriate time and place of this medicine for disadvantaged because of the estrangement or the absence of health service is also an unbearable reality^{13,14}.

Considering these binding factors, the therapy by plants appears as a less expensive and more easily accessible

Table 1: summary of the chemical characterization.

Chemical groups	Results
Steroidic Esters and/or triterpenic	+
Flavonic Aglycones	+
Aglycone of the Antracenosides	+
Carotenoids	+
Steroidic Glycosides and/or triterpenic	+
Flavonoids	+
Coumarine	-
Leucoanthocyanes	+
Antracenosides	+
tannins	+
Saponosides	+
reducer compound (sugars)	+

+: present; -: absent

alternative, if its therapeutic efficiency and its harmlessness are scientifically proved.

Indeed, the recourse to healing plants for the treatment of various affections is the own of the populations of developing countries and that of Burkina Faso in particular. Approximately four hundred and twenty seven (427) species of plants are used for medical purposes by the populations of the "plateau Mossi" in Burkina Faso for the treatment of various affections, including the gastro-intestinal parasitosis¹⁵.

This traditional medicine that gave better results in the treatment of some diseases where the modern medicine had relative failures¹⁶, is at present recognized by the national authorities in Burkina Faso where it is integrated in some policies of the health system¹⁷.

In the research for new biologically active molecules from healing plants for the development of phytomedicine against gastro-intestinal parasitosis, many authors in Burkina Faso have conducted studies on the anthelmintic properties of some plants. Traore et al¹⁸ showed that *Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii* three plants used in the traditional medicine have anthelmintic effects on the adult worms of *Haemonchus contortus*. Gnoula et al¹⁹ on *Balanites aegyptiaca* and Kaboré²⁰ on *Anogeissus leiocarpus* and *Daniellia oliveri* showed the anthelmintic effect of these plants.

In Africa and in Europe we can also quote authors as Githiori et al²¹, Hounzangbe-Adote et al²², Okombé²³, Sreejith et al^[24] who realized studies on plants with anthelmintic properties.

In this context of research for new active biomolecules the present study was done to test the anthelmintic efficiency of *Acacia nilotica* var. *adansonii* (Guill & Perr). *O Ktze* (Mimosaceae), plant used in traditional medicine in Burkina Faso for the treatment of intestinal parasites. The barks and pods of this plant are used in rural areas to treat diarrhea, the verminoses and the gastritis²⁵. Some of the antiparasitic properties in particular the molluscicidal effects²⁶ and the antibacterial properties²⁷ were already proved.

This study aims to evaluate the *in vitro* anthelmintic properties of the aqueous extract of the trunk barks of *Acacia nilotica* var. *adansonii*.

MATERIALS AND METHODS

Chemicals

The Phosphate Buffered Saline (PBS) / Buffer Phosphates Salt marsh was used as negative control whereas a solution of levamisole (1 % w: v) prepared with the solution of PBS was used as positive control

Plant material

Trunk barks of *Acacia nilotica* var. *adansonii* were collected in April 2013 at Koupèla, city located at 140 km in the east of Ouagadougou (Burkina Faso). Plant sample was identified by the "Herbier National du Burkina" of the Centre National de la Recherche Scientifique et Technologique (CNRST) where a voucher specimen was deposited under number HNBU00210.

Animal material

Evaluation of the *in vitro* anthelmintic effect of the aqueous extract of trunk barks of *Acacia nilotica* var. *adansonii* was realized on eggs and adult worms of *Haemonchus contortus*. These worms were harvested on the stomach of naturally infected goats or sheep. The stomach were bought from the refrigerated slaughterhouse of Ouagadougou, conditioned in an icebox and forwarded in the laboratory.

Collection of the adult worms of *Haemonchus contortus*

The adult worms collected after a longitudinal section of the stomach were carefully sorted and washed successively in the distilled water to clear them of fragments of feces. They were then placed in a Petri dish containing a solution of PBS (Phosphate Salt Buffer, pH: 7.2) then immediately used to realize the biological test

Collection of the eggs of *Haemonchus contortus*

The eggs of *Haemonchus contortus* were collected according the technique described by Jabbar et al²⁸. The adult worms were slightly crushed in a mortar with a porcelain pestle to free eggs. The obtained solution was filtered through sieves with stitches of various dimensions (1mm and 100 µm). Then, a sieve with stitches of 38 µm allowed retaining freed eggs. Eggs were got back by rinsing of the sieve with some distilled water. The obtained solution of eggs was adjusted to approximately 1000 eggs / mL.

Preparation of extract and phytochemical analysis

The plant material harvested were cleaned, dried in the shade under ventilation, and then powdered using a mechanical grinder. The powder was used to prepare extracts for phytochemical and anthelmintic studies.

For extract preparation, 200 g of the of trunk barks was placed in a flask (1l capacity) with distilled water (700 ml). After homogenization and maceration during 24 hours, the mixture was filtered through of a nylon fabric, then the filtrate centrifuged at 2000 tours / min for 10 min. The extracts were collected and dry-evaporated in a steam room ventilated in the temperature of 50°C during 72 hours. The dry extract was put in flasks in high-density polyethylene and kept in a freezer.

The tests of chemical characterization were realized in test tubes according to the method described by Cieulei²⁹. The principle of this method is based on the capacity of certain functional groups of active phytochemical substances to react with specific or general reactive to give reactions

Table 2: mortality rate of the adult worms of *Haemonchus contortus* after a contact of increasing concentrations of the aqueous extract of *Acacia nilotica* var. *adansonii*.

Extract: <i>Nilotica</i> (mg/mL)	Mortality rate (T.M %, n=5)		
	Time of contact		
	4h	6h	24h
0.0	-	-	14.81 ± 0.57
0.1	-	-	29.60 ± 5.20
1.0	-	-	59.26 ± 5.09
3.0	-	-	62.97 ± 5.23
10.0	-	-	77.75 ± 0.05
15.0	-	-	88.89 ± 0.02

Table 3: mortality rate of adult worms of *H. contortus* after a contact with the levamisole.

Tested product: Levamisole (mg/mL)	Mortality rate (T.M %, n=5)		
	Time of contact		
	4 h	6 h	24 h
0	-	-	14.81±0.57
0.066	-	-	33.33±0.1
0.6	-	11.11±0.57	44.44±0.57
2	-	22.22±0.57	66.66±0.57
6.66	11.11±0.57	33.33±0.1	88.88±0.0
10	66.66±0.1	77.77±0.57	100±0.57

(coloring, precipitation) characteristics. Phytochemical groups which researched were particularly polyphenols, tannins, flavonoids, terpenoids.

In vitro anthelmintic activity

Test of inhibition of the mobility of the adult worms

The adult worms of *Haemonchus contortus* were placed in Petri dish containing the PBS, at the rate of 3 worms by Petri dish. The solutions of the extract dissolved in the PBS in the increasing concentrations of 0.1; 1.3; 10 and 15 mg/mL were added in the Petri dish, in a total volume of 3 mL. The parasites were incubated at 37 °C during 24 hours. Motility and the survival of worms were observed by means of the optical microscope at 2; 4; 6 and 24 hours.

After 6 hours of incubation, worms treated with the extract of plant and the levamisole were dipped back during 30 min in the solution of PBS to observe the possible resumption of the motility. The number of worms died after the exposure in the extract was estimated at 4 hours, 6 hours and 24 hours and the mortality rate of worms (TM) for each of the concentrations of the extract were calculated by using the following formula:

$$TM(\%) = \frac{\text{Number of dead worms}}{\text{total worms put in the Petri dish}} \times 100$$

The test was realized in three (3) replications and repeated five (05) times for each of the concentrations of the extract.

Eggs hatching inhibition test

This test was performed according to Coles et al.³⁰. A volume of 0.1 mL of the eggs suspension adjusted to approximately to 1000 eggs / mL were put in the wells of 24-wells plate. In every well, it was added 1.9 mL of extract of the trunk bark of *Acacia nilotica* var. *adansonii*

in the final concentrations of 0.1; 1; 3; 10 and 15 mg / mL. Negative and positive controls consisted respectively to PBS and levamisole solution (levamisole in 1 % w: v in the PBS) were constituted.

Then, the closed plates were incubated in the temperature of 25°C for 48 hours. At the end of incubation time a drop of formalin was added in every well to stop the evolution of eggs. Then, staying eggs were counted in the microscope (Olympus BH-2, Optical Co. LTD, Japan) at 40 x. The test was realized in 3 triplicates for each of the concentrations and repeated independently five times. The rate of hatching (TE) eggs for each of the tested concentrations was estimated according to the following formula:

$$TE(\%) = \frac{\text{Number of remaining eggs}}{\text{Number of eggs put in the well}} \times 100$$

Statistical analysis

The statistical data concerning the average percentages of the mortality rates (TM) and the inhibition of the hatching of eggs (IEO) were analyzed with the software GraphPad Prism 5.0. (Reference). The obtained results were expressed in the form of mean ± Standard Average Error (m ± E.S.M.). The variations were considered as significant for a probability of error (p) lower than the risk granted 0.05 (p<0.05).

RESULTS

Phytochemical Analysis

The tests allowed detecting certain phytochemical families present in the dry extract of powder of the bark of trunk of *Acacia nilotica* var. *adansonii*. The results are given by the table 1.

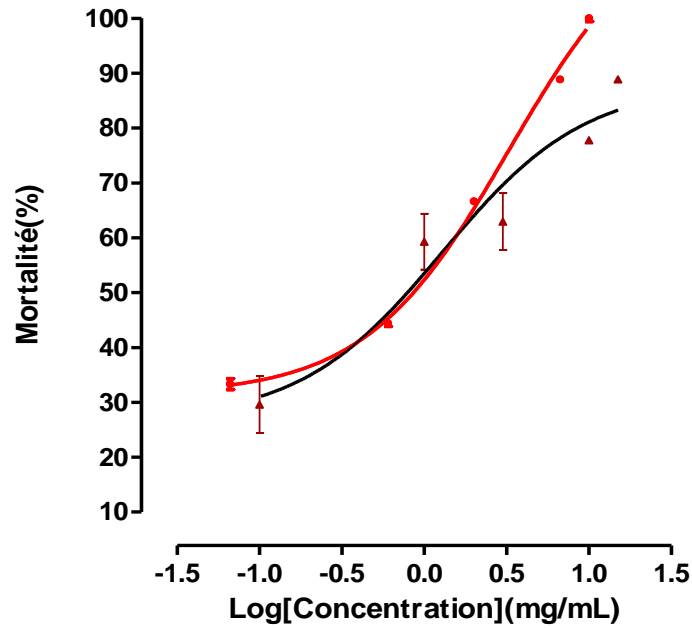
Effects on the adult worms of haemonchus contortus.

At the 4th and 6th of observation, some worms presented a stiff posture to the concentrations of 10 mg / mL and 15 mg / mL. Besides the total immobility of worms noticed, the extract induced mortality of the worms after 24 hours (table 2). And this lethality was proportional to the concentrations. With the levamisole (table 3), the death of worms was noticed at already 4 hours and 6 hours of incubation in the concentrations 2mg/mL; 6.66mg/mL and 10mg/mL. The results are presented in table 2 and 3 below. Table 3: mortality rate of adult worms of *H. contortus* after a contact with the levamisole. The analysis of the data by the software prism allowed to determine the lethal concentration of 50% of adult worm death at 24h for each extract (figure 1).

Through analysis of figure (1), the values of the lethal concentration of 50 % death (LC₅₀), corresponding to the death of 50 % of adult worms are 1.28 mg / mL and 3.25 mg / mL respectively for the aqueous extract and the levamisole.

Effects on the hatching of eggs of haemonchus contortus.

At the end of the 48 hours of incubation, non-hatched eggs in every well were counted to establish the inhibition of the egg hatching. The percentage of the inhibition of egg hatched was increased with the increased concentrations of the extract. This evolution was not noticed with the levamisole. Non hatched eggs were also observed with



● Lévamisole (LC₅₀=3,25mg/mL); ▲ Acacia nilotica (LC₅₀=1,28mg/mL)

Figure 1: Dose-effect of the aqueous extract of the trunk barks of *Acacia nilotica* var. *adansonii* and of the levamisole on the mortality of the adult worms of *H. Contortus* after 24 hours of exposure.

Table 4: percentage of inhibition of hatching eggs of *H. contortus* by concentration of aqueous extract of *A. nilotica* after incubation over 48 hours.

Extract	Concentrations					
	0mg/ml (PBS)	0.1mg/ml	1mg/ml	3mg/ml	10mg/ml	15mg/ml
Trunk bark <i>A. nilotica</i>						
P.I (% , n=5)	18.10±1.16	93.84±2.9 ^a	96.67±1.1 ^b	96.92±2.7 ^b	98.72±0.6 ^b	99.48±0.72 ^b

A significant difference ($p < 0.05$) was noticed with the various concentrations and the maximal efficiency (E_{max} = 93.84 %) was reached at 0.1 mg/ml concentration of extract

negative control (PBS). The results are presented in tables 4 and 5.

DISCUSSIONS

The extract of study was prepared according to the ethno-pharmacological indication. Thus, the aqueous maceration was adopted as model of extract to be estimated.

The results obtained in this study indicates a considerable anthelmintic activity of the aqueous extract of the trunk bark of *Acacia nilotica* var. *adansonii*, due to the inhibitory effect observed as well on the adult worms as on the eggs of *H. contortus*.

On the adult worms, the value of the lethal concentration 50 % (LC₅₀) of 1.28 mg / mL is lower than the LC₅₀ value obtained with levamisole (LC₅₀= 3.25mg / mL). However, the levamisole presented a better kinetics and efficiency of action with 100 % of adult worms died at 10mg / mL in comparison with the extract of *A. nilotica* that induced 88.89 % of mortality at 15mg / mL.

The anthelmintic activity of the seeds of *Acacia nilotica* var. *adansonii*, on the adult worm of *H. contortus*, has been not significant³². *In vitro* tests on this kind of parasites often realized with the L1 or L3 types of larvae.

Nevertheless the concentration (LC₅₀) of 5.03 mg / mL which caused 50 % of death of adult worms of *H. contortus* obtained with the extract of *Saba senegalensis* by Belemliiga³³. That consolidates the result obtained with the trunk bark of *Acacia nilotica* var. *adansonii*.

The inhibition of the hatching of eggs of *H. contortus* obtained with the levamisole is very contrasted because going down from 12.72 % inhibition at the concentration of 10mg / mL to 0 % of inhibition at the concentration of 15mg / mL without any larvae in the medium. The high anthelmintic power of this medicine is well known and its effect in our study could be explained by the lysis of the eggs. In the PBS, the 18.10 % of noticed inhibition would result from the absence of optimal condition favoring the maturation of eggs to allow their hatching³⁴ or of their bad state.

Concerning the extract of the trunk bark of *A. Nilotica*, a 93.84 % rate of eggs hatching inhibition was observed with the concentration of 0.1mg / mL. That indicates a powerful ovocidal activity of the extract. Proximate conclusions had been reported by Egualé et al³¹ who obtained 50% rate of eggs hatching inhibition with the concentration of 0.87mg / mL for the aqueous extract of the seeds of *Acacia nilotica*

Table 5: percentage of inhibition of hatching eggs of *H. contortus* by concentration of levamisole after incubation over 48 hours.

Product	Concentrations					
	0mg/mL (PBS)	0.066mg/mL	0.6mg/mL	2mg/mL	6.66mg/mL	10mg/mL
P.I (% , n=5)	18.10±1.16	22.72±0.57	22.72±1.16	12.72±0.54	12.72±0.54	00±0.57

var *adansonii* Wild and Del. In Pakistan, Zafar et al³⁵ obtained 50% rate of eggs hatching inhibition at the concentration of 0.51mg / mL for the same seeds of Locust tree *Acacia nilotica* var. *nilotica* on eggs of *H. contortus*. This anthelmintic effect of the trunk bark of *Acacia nilotica* var. *adansonii* (Guill & Perr) is attributable to the phytochemical groups that it contains. The phytochemical screening of the aqueous extract trunk barks revealed the presence of flavonoids, tannins, anthocyanosides, steroidal glycosides so as Nacoulma¹⁵ results. Triterpéniques, saponins, reducer compound was also identified. These results are comparable to those obtained by Bushra et al³¹ on the main metabolites of the plants with tannins. The phytochemical groups which identified indicate a possible anthelmintic property of the trunk barks of *Acacia nilotica* var. *adansonii*. For many authors, the condensed tannins, would act by settling on the cuticle of the adult worms or on the girdle of larvae³⁶, causing then a disturbance of their mobility. It would also cause a change of the enzymatic process by settling on enzymes secreted by worms³⁷. Even if for the moment, their modes actions on helminthes remain underestimated, saponosides compounds³⁸ as well as terpenoides³⁹ identified as having anthelmintic properties, could contributed to the anthelmintic effect of the bark of trunk of *Acacia nilotica* var. *adansonii*.

CONCLUSION

The results from this study revealed that the aqueous extract of the trunk bark of *Acacia nilotica* var. *adansonii* (Guill & Perr). O Ktze shown *in vitro* anthelmintic activity against adult worms and eggs of *Haemonchus contortus*. The traditional use of this plant by traditional healers and pastoralists as anthelmintic seems to be justified. However, it would be necessary to achieve acute toxicity study and *in vivo* parasitological studies.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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