

IN VITRO NEMATOCIDAL EFFECTS OF AQUEOUS EXTRACTS OF LEAVES AND BARK OF KHAYA SENEGALENSIS AND AZADIRACHTA INDICA ON THE MORTALITY OF ADULTS OF HAEMONCHUS CONTORTUS, COBB, 1899 (RHABDITIDA, TRICHOSTRONGYLIDAE)

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ABSTRACT

The present study aimed to evaluate the in vitro effects of aqueous extracts of leaves and bark of Khaya senegalensis and Azadirachta indica on the mobility of Haemonchus contortus adults in Senegal. The outcome is that the uncontrolled use of synthetic anthelmintics has led to the emergence of resistance. Therefore, to limit the damage caused by intestinal parasites such as *H. contortus* to the profitability of small ruminant farming systems, phytotherapy appears to be the only possible solution. To this end, live parasites from sheep and goats sampled at the Medina abattoir were placed in Petri dishes at a rate of 5 worms per dish. A 3mL volume of the drug preparation (0.2g/mL) to be tested and/or the control solution was added. Phosphate Buffer Saline (PBS) was used as the negative control and Albendazol as the positive reference substance (positive control) at 25mg/mL concentration in this series of tests. Evaluating the anthelmintic activity of neem and khaye leaf extracts gave interesting results. Thus, inhibition rates of and rapid death in affected ruminants and especially lambs 100% were reached after 18 hours under the effect of neem and 20 hours in contact with khaye. Similarly, the activity of the bark extracts from these plants produced inhibition rates of one hundred per cent at around 18 hours for the khaye and 22 hours for the neem. In addition, survival analysis using the log rank test showed a highly significant difference between natural death and death caused by aqueous leaf extracts. These extracts could be judicious alternatives to chemical drugs against hemonchosis in small ruminants in endemic areas such as Africa.

Keywords: Haemonchus contortus, Small ruminants, In vitro, Nematicide, Azadirachta indica, Khaya senegalensis, Leaf, Bark

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1. INTRODUCTION

Haemonchus contortus is the most abundant helminth in the abomasum of small ruminants. It is responsible for hemonchosis, which leads to significant production losses in livestock systems (Williamson 2014). Indeed, heavy loads of this hematophagous nematode can cause severe anaemia and rapid death in affected ruminants and especially lambs (Hoste et al. 2006; Arsenopoulos et al. 2021). Therefore, the focus of parasite control is based on a combination of chemo-therapeutic measures, grazing management, feeding, biological control, vaccination and ethnoveterinary treatment (Hoste et al. 2006; Dkhil 2013). However, modern control programmes have relied heavily on the use of synthetic anthelmintics. These have drawbacks, such as the development of resistance (Mumed et al. 2022). The species most affected by these AMR (antimicrobial resistance) phenomena is *H. contortus*. It is known to be highly adaptable and to develop multiple forms of resistance. Resistance to benzimidazole derivatives is one of the most worrying cases, as it is one of the most widely used anthelmintics, mainly because of its accessibility and age on the market (Tasawar et al. 2010; Santos et al. 2012; Tak et al. 2013; Niguse et al. 2014). In addition, no national survey of anthelmintic resistance has yet been carried out in Senegal. However, studies conducted by Sambe (2015) reported the presence of the allele responsible for *H. contortus* resistance to albendazole. As an alternative, research is being carried out to identify local plant products with potential anthelmintic properties (Hoste et al. 2006). Numerous studies have been published on the potential





nematocidal activity of local plants against *H. contortus* and related trichostrongylid parasites. These include *A. indica* (Abu Hawsah et al. 2023) and *K. senegalensis* (China et al. 2016), which have been shown to have nematocidal activity. The aim of this study was to evaluate the in vitro effect of aqueous extracts of the leaves and bark of these plants on the mortality or inhibition of *H. contortus* adults.

2. MATERIALS AND METHODS

This study was conducted entirely in the Entomology and Acarology Laboratory of the Department of Animal Biology at Cheikh Anta Diop University (UCAD) in Dakar. The objective was to assess the anthelmintic efficacy of aqueous extracts from the leaves and bark of *A. indica* (neem) and *K. senegalensis* (khaya) on adult *H. contortus*.

2.1. Sampling

Given the objective of this study, only the coagulates and a few centimeters of the small intestine from small ruminants were collected. For each sampling session, three random offal samples from sheep and/or goats were obtained from the Medina abattoir. Each sample was placed in a bag labeled with the host species (sheep or goat) and transported to the laboratory in a cooler.

2.2. Helminthological Autopsies of Offal

The dissection was based on the methods of Graber and Perrotin (1983). For each sample, the viscera (abomasum) were cut open lengthwise, and the contents were carefully rinsed under a gentle stream of water. The worms were gently collected using forceps and then immersed in Phosphate-Buffered Saline (PBS) for the in vitro efficacy test of the aqueous extract of *A. indica* and *K. senegalensis* leaves.

2.3. Preparation of the Aqueous Extract of the Leaves and Barks of A. indica and K. senegalensis

The leaves of both plants were harvested after sunset, while the barks were collected during the day from the UCAD botanical garden. The leaves were thoroughly washed with water, and the bark was dried in a dust-free environment, away from direct sunlight, for 10 to 15 days before being ground into a fine powder using an electric grinder. The powder from each plant was stored in labeled, airtight jars until use.

Extraction was performed by macerating the plant powder in distilled water at a ratio of 50 g of powder to 250mL of distilled water. The mixture was mechanically stirred for 48 hours, then filtered using a tea strainer followed by a finer filter cloth. The resulting filtrate was used on the same day for the tests. The remaining extract was stored in the refrigerator for future use.

2.4. In vitro Tests

A total of 108 live adult *H. contortus* worms were used in this series of tests. Five worms were placed in each Petri dish, which contained 3mL of the drug preparations to be tested and/or the control solution. This technique was inspired by Houngnimassoun (2020). Phosphate Buffer Saline (PBS) was used as the negative control and Albendazol as the positive reference substance (positive control) at a concentration of 25mg/mL in this series of tests.

These different concentrations of extract were selected following preliminary tests carried out on certain gastrointestinal nematodes of small ruminants, which showed inactivity at doses of less than 20mg/mL. The mobility of the worms was observed at one-hour intervals for 24 hours. As an observation criterion, any worm showing continuous total immobility for 15 seconds was suspected of being dead and was carefully removed from the Petri dish and placed in a solution of PBS or distilled water after 5 minutes to ensure that the worm was immobile.

2.5. Data Analysis

The results of the inhibition of the mobility of adult *H. contortus* worms to aqueous extracts of neem and khaye were entered and cleaned using Microsoft Excel 2021 and survival analysis to study the occurrence of helminth mortality after exposure to the substances of interest (biocide) using R. 4.3.1 software (R Core team 2023). Survival analysis is a statistical method used to analyze the time it takes for a particular event to occur. Here, that event is the mortality of the worm exposed to the drug products. The log-rank test was used to assess this difference. This test is a statistical method for comparing the survival curves of two or more groups. The significance of the differences is assessed at the threshold of 5.

3. RESULTS

Table 1 shows the mobility inhibition rates of *H. contortus* adults subjected to aqueous extracts of the leaves and bark of *A. indica* and *K. senegalensis*. The effects of aqueous extracts of A. indica begin to appear 6 hours after exposure, at a rate of 10% for leaves and 23.33% for bark. These effects gradually increased, reaching 100% at 18 hours for the leaves and 24 hours for the bark. Finally, *A. indica* leaves appear to be more effective than bark at rapidly reaching 100%, although both reach this level, but bark reaches this effect later at 24 hours.



Plant/part	Dose/Box	Time (Hours)							
		3	6	9	12	15	18	21	24
A. indica (Leaf)	0.2g/mL	0	10	16.67	20	56.67	100	-	-
A. indica (Bark)	0.2g/mL	0	23.33	43.33	56.67	63.33	63.33	96.67	100
K. senegalensis (Leaf)	0.2g/mL	10	30	63.33	66.67	66.67	93.33	100	-
K. senegalensis (Bark)	0.2g/mL	0	16.67	46.67	76.67	83.33	100	-	-
Positive control (Albendazol)	0.025g/mL	100	-	-	-	-	-	-	-
Negative control (PBS 10X; pH 7.4)	3mL	0	3.33	13.33	13.33	43.33	60	70	73.33

The effects of aqueous extracts of K. senegalensis began to appear 6 hours after exposure, with an initial level of 10% for leaves and 16.67% for bark. These effects progressively increase to 100% at 18 hours for the barks and 21 hours for the leaves. Definitively, the leaves and bark of K. senegalensis show similar results in terms of maximum efficacy, but the bark seems to be more effective earlier, reaching a percentage of 100% at 18 hours.

None of the plant extracts achieved efficacy as quickly as albendazole, which showed immediate action at 3 hours with 100% efficacy. However, the negative control shows that a weak effect is possible even without treatment, but plants and albendazole are more effective.

3.1. Effects of Aqueous Extracts from the Leaves of K. senegalensis and A. indica plants on the Mobility of H. contortus

The survival analysis shown in Fig. 1 revealed that the aqueous extracts from the leaves of the two plants had a significant effect on the mobility of the parasites over 24 hours. The log-rank test gave a highly significant (P<0.0001), indicating that the aqueous extracts of the plants had a significant impact on the mobility of small ruminant parasites. This p-value suggests that the differences observed between the three groups are probably not due to chance.

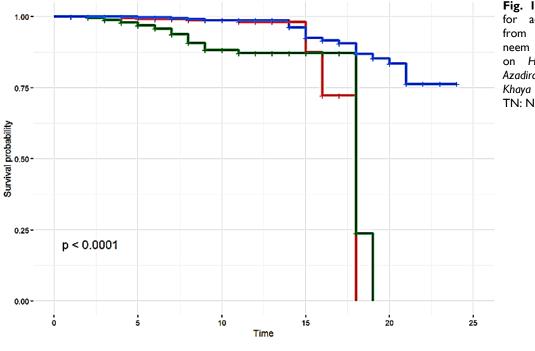
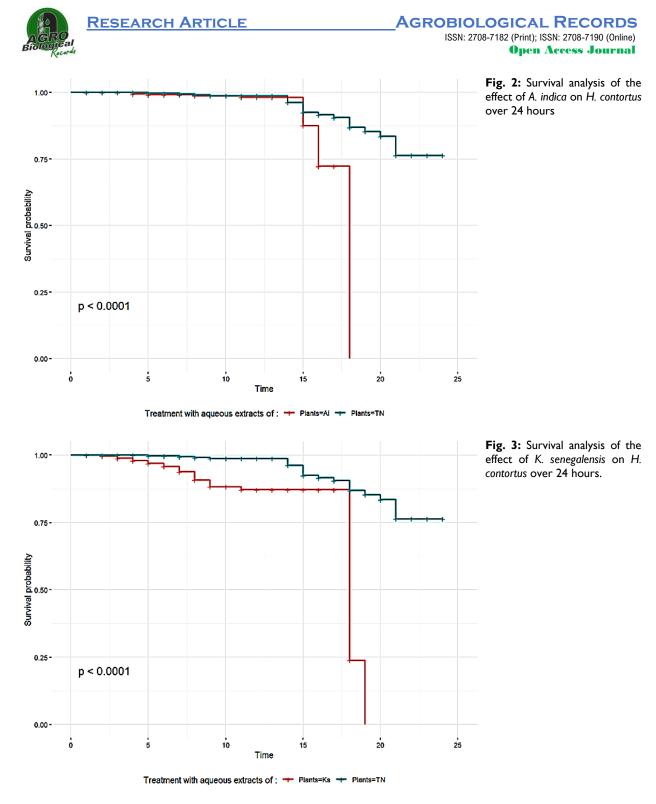


Fig. I: Survival curves for aqueous extracts from the leaves of neem and khaye plants on H. contortus. Ai: Azadirachta indica; Ks: Khaya senegalensis and TN: Negative control.

Treatment with aqueous extracts of : 🕶 Plants=AI 🕶 Plants=Ks 🕶 Plants=TN

Fig. 2 demonstrates a significant effect of aqueous extracts of neem leaves on parasite inhibition compared to the natural inhibition group (TN), as indicated by the log-rank test (P= 3e-08).

A. indica appears to have a stronger and faster nematocidal effect compared to K. senegalensis (Fig. 3). The control group (TN) shows natural mortality that progresses more gradually. The survival analysis, confirmed by a log-rank test with a p-value of 1×10^{-4} , demonstrates the significant efficacy of these plant extracts against Haemonchus contortus adults in vitro.



3.2. Effects of Bark Extracts from K. senegalensis and A. indica plants on H. contortus

According to Fig. 4, the aqueous extracts of the bark of the two plants reached a total inhibition effect before 24 hours. Parasite survival probabilities were zero with *K. senegalensis* bark at around 18 hours and with *A. indica* bark at around 22 hours. The log-rank test revealed an extremely low (P<0.0001), indicating that this difference between the products tested is highly significant and unlikely to be due to chance. So, in addition to leaves, barks could also represent potentially effective alternatives to anthelmintics.

The survival curve showing the effect of the aqueous extract of *A. indica* bark (Fig. 5) indicates an inhibition significantly (P<0.001) different from that of the negative control according to the log-rank test. Similarly, the anthelmintic effects of *K. senegalensis* bark (Fig. 6) were also significantly (P<0.05) different from those of PBS (negative control).

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10

Time

15

20

25



1.00

0.75

Survival probability

0.25

0.00

ò

p < 0.0001

5

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Fig. 4: Survival curves for aqueous extracts of neem and khaye plant barks on *H. contortus*. EAi: Azadirachta indica bark; EKs: *Khaya senegalensis* bark and TN: Negative control.

Fig. 5: Survival analysis of the effect of *A. indica* bark on *H. contortus* for 24 hours.

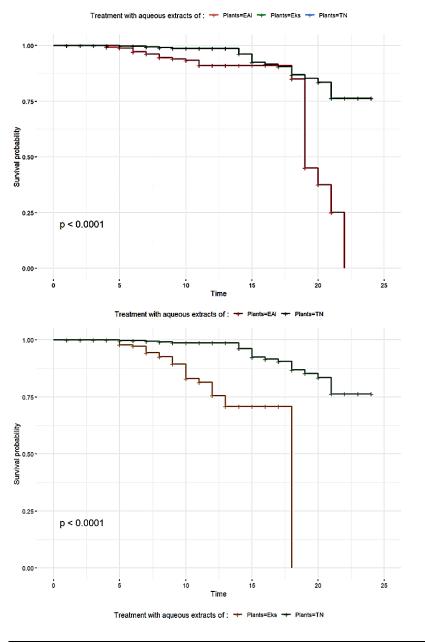


Fig. 6: Survival analysis of the effect of *K.* senegalensis bark on *H. contortus* for 24 hours.



4. **DISCUSSION**

Hemonchosis is a common and serious disease that affects the abomasum of ruminants in many regions of the world (Mehlhorn 2008). Conventional anthelmintics, such as benzimidazoles, pyrantel, and ivermectin, are frequently used to treat these parasitic infections (Kaplan and Vidyashankar 2012). Although they are effective against a wide range of nematodes and generally well tolerated by animals, their prolonged use has led to the emergence of significant resistance among parasite populations, thus reducing their effectiveness. In addition, these drugs can be costly for farmers and may have potential environmental impacts due to their accumulation in soil and water (Leathwick and Besier 2017).

In response to these challenges, phytotherapy emerges as a promising alternative (Büchi and Lüthi 2017; Ahuir-Baraja et al. 2021; Hawadak et al. 2022; Ranasinghe et al. 2023). Plants such as *A. indica* and *K. senegalensis* are being studied for their anthelmintic potential, offering a natural alternative to chemical treatments with additional benefits in terms of safety and sustainability. Resistance to anthelmintics in the causative agent of hemonchosis has limited the control of nematodes, highlighting the need to find alternatives, such as phytotherapy. This approach may offer safe, cost-effective products for farmers that are effective against gastrointestinal strongyles (Jato et al. 2022).

Recent studies confirm this perspective. Furthermore, the study by Binta et al. (2022) demonstrated that methanolic extracts of leaves, stem bark, and roots of A. indica have significant nematocidal activity against *H. contortus*, suggesting their potential as an alternative to conventional anthelmintics. Another study published by Zabre et al. (2024) revealed that ethanolic extracts of neem leaves result in high mortality of adult H. contortus and significant inhibition of egg hatching, indicating notable efficacy against this parasite. These results reinforce the idea that plant extracts, particularly from Azadirachta indica, offer a promising alternative to traditional chemical treatments against gastrointestinal nematodes in small ruminants.

By the same, the objective of this study was to evaluate the comparative anthelmintic power in vitro of raw aqueous extracts of *A. indica* and *K. senegalensis*, commonly found in Senegal, compared to the chemical drug albendazole on the mobility or mortality of nematodes, *H. contortus*, in small ruminants. The evaluation of the anthelmintic properties of neem and khaye leaf extracts yielded interesting results. Inhibition rates of more than a hundred percent were achieved after 18 hours under the influence of neem and 20 hours in contact with khaye.

Moreover, utilizing the log rank test for survival analysis, it was found that the death caused by leaves aqueous extracts was highly significant compared to natural death. The abundance of methanol, acetone, and tannins in these plants makes them effective at all three levels of parasite development (egg, larval and adult) (China et al. 2016). OOAS (2013) reported that these plants have anthelmintic properties against intestinal parasites.

Similarly, the bark extracts activity of these plants resulted in a 100% inhibition at about 18 hours for khaye and 22 hours for neem. A significant difference was observed between the negative control and the tested products in the survival analysis using the log rank test. The results of Ademola et al. (2009) and China et al. (2016) both indicate that khaye bark extracts have a significant impact on sheep's digestive strongles in vitro/vivo.

Neem in the form of seeds or leaves has a significant effect on strongles in animals (Hordegen et al. 2006; Jamra et al. 2015; Abdulai et al. 2023). The comparison of the effects of aqueous extracts of neem leaves and bark and aqueous extracts of khaye leaves and bark also revealed a significant difference. Only both parts of the neem plant showed this significant difference in efficacy. However, the efficacy of both parts of the khaye plant is not statistically significant. It is also important to note that plant extracts showed a significant effect after 3 hours, which is close to the effectiveness of the best anthelmintics available. According to OOAS (2013), the most effective synthetic products act after 3 hours.

5. CONCLUSION

Based on analysis of the results obtained, aqueous extracts of the leaves and bark of *A.indica* and *K. senegalensis* appear to have effective anthelmintic properties that can be used as an alternative to chemical products. In addition, these extracts showed promising in vitro anthelmintic activity against *H. contortus*, confirming and encouraging the traditional use of these plants as anthelmintics. The efficacy of extracts increases with exposure time. In addition, for valid scientific arguments on the use of local veterinary phytotherapy, it would be necessary to study the in vitro effect of these plants on egg hatching and larval development, as the in vivo effect on grazing in order to control the level of infestation.

Author's Contribution

Boubacar Cissokho: Conceptualized the study, conducted the investigation, developed the methodology, performed formal analysis, and wrote the original draft. Babacar Souleymane Sambe: Administered the project, validated the findings, contributed to the conceptualization, and reviewed and edited the manuscript. Toffène Diome: Provided supervision, resources, and validation, and reviewed and edited the manuscript. Mama Racky

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Ndiaye: Reviewed and edited the manuscript. Mbacké Sembène: Supervised the project, provided resources, and reviewed and edited the manuscript.

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