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Evaluation of *in Vitro* Antiparasitic Activity Against *Haemonchus Contortus* of Three Forage Plants From The Flora of Côte D'ivoire

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Abstract
Intestinal nematodes continue to pose a major constraint in small ruminant production. Across the globe, each country is actively seeking innovative, cost-effective, and environmentally sustainable strategies to improve control of these parasites. Among the promising alternatives, livestock-consumed plants are increasingly being explored for their potential in managing gastrointestinal nematodes in domestic ruminants. This study aimed to evaluate the anthelmintic potential of three forage plants from the local flora for the control of digestive tract nematodes in small ruminants. To achieve this, adult worms of the nematode *Haemonchus contortus* Rudolphi (Trichostrongylidae) were incubated in Petri dishes and exposed for a specific duration to hydromethanolic leaf extracts of *Albizia adianthifolia*, *Ficus lutea*, and *Morus mesozygia*. The *in vitro* anthelmintic assays revealed that *F. lutea* and *A. adianthifolia* exhibited adulticidal activity against *H. contortus* worms. Notably, *A. adianthifolia* demonstrated the highest efficacy, causing 100% mortality of adult worms after 24 hours of exposure. These findings underscore the ecological value of forage plants in the sustainable control of gastrointestinal nematodes affecting sheep and goats.

Keywords
Albizia adianthifolia, *Ficus lutea*, *Haemonchus contortus*, anthelmintic activity

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INTRODUCTION
Live stock farming of small ruminants, particularly goats and sheep, plays a significant socio-economic role across many regions of the world, especially in Africa [1]. However, the health of these animals is continually threatened by gastrointestinal parasitic infections, primarily caused by helminths [2]. These infections lead to stunted growth, reduced productivity (milk, meat, wool), reproductive disorders, and, in severe cases, death resulting in substantial economic losses for livestock farmers [2,3]. Traditionally, parasite control has relied on synthetic anthelmintics. Although effective, their intensive and often indiscriminate use has rapidly led to the development of parasite resistance, rendering treatments increasingly ineffective [4,5,6]. Moreover, growing consumer concerns regarding drug residues in animal products

and the need to promote more sustainable and environmentally friendly livestock systems are driving interest in alternative approaches [7]. In response to this multifaceted challenge, research is increasingly exploring natural and innovative solutions. One promising avenue lies in the integration of forage plants with intrinsic anthelmintic properties. These plants, often accessible and already part of the animals' environment, could not only support parasite management but also enhance herd nutrition and overall health while reducing dependence on chemical treatments and their associated drawbacks. Several studies have highlighted the anthelmintic potential of such plants, particularly forage legumes rich in condensed tannins [8,9]. According to [10], the consumption of these plants by ruminants slows the progression of parasitic infestations, thereby decreasing the need for synthetic anthelmintics. In Côte d'Ivoire, forage species are distributed across various botanical families, including Moraceae and Leguminosae (Fabaceae), which, according to [11], are among the most representative of the local flora. Species from these two families are Dicotyledons unlike those from the Poaceae family (Monocotyledons) and are noted for their richness in bioactive compounds active against a range of pathogens. The objective of this study is therefore to highlight the potential role of local forage plants in the control of gastrointestinal helminths in small ruminants. Specifically, it aims to demonstrate the anthelmintic activity of three local forage species against adult worms of the nematode *Haemonchus contortus*, which is one of the predominant parasites affecting goats and sheep in Côte d'Ivoire.

MATERIALS AND METHODS

Preparation of crude plant extracts

The plant material used in this study consists exclusively of the leaves from three forage species: *Albizia adianthifolia* (Schumach.) W. Wight (Fabaceae) and *Morus mesozygia* Stapf (Moraceae), both harvested in Abidjan, and *Ficus lutea* Vahl (Moraceae), collected in Pacobo, a Sub-prefecture located at the southern tip of the Baoulé "V" savannah. These three plant species were selected based on preliminary *in vitro* assays demonstrating biological activity of their hydro-methanolic extracts against the nematode *Haemonchus contortus*. Specifically, the extract of *Morus mesozygia* exhibited inhibitory effects on egg hatching, while *Albizia adianthifolia* and *Ficus lutea* showed efficacy against L1 and L2 larval stages.

The collected leaves were air-dried in a temperature-controlled room (18 °C) for one to two weeks to minimize the degradation of heat-sensitive phytochemicals commonly affected by direct solar exposure. The dried material was then ground manually using a mortar and pestle to obtain a fine powder. Extraction was carried out by maceration in 90% methanol at a 1:10 ratio (10 g of plant powder per 100 mL of solvent) for 24 hours under continuous mechanical stirring at ambient temperature. Following filtration, the extracts were concentrated using a rotary evaporator set at 40 °C. The resulting aqueous residue, obtained after methanol evaporation, was further dried in an oven at 40 °C over a period of three days.

Collection of adult *Haemonchus contortus* worms

Adult specimens of *Haemonchus contortus* Rudolphi (Trichostrongylidae) were isolated from the abomasums of naturally infected sheep. The abomasal tissues were sourced from butchers operating at the Coco Service livestock market, located near Nangui Abrogoua University in Abidjan. Upon collection, longitudinal incisions were made in each abomasum to expose its contents. When *H. contortus* was visually confirmed, the abomasums were immediately immersed in polypropylene containers filled with phosphate-buffered saline (PBS, pH 7.2) and transported to the laboratory for further examination.

Anthelmintic tests

Initially, 40 mg of dried extract residue from each plant species were homogenized in 250 µL of dimethyl sulfoxide (DMSO) using a vortex mixer. From this homogenate, 10 µL were diluted 80-fold with distilled water to yield a final concentration of 2 mg/mL. This stock solution was subsequently subjected to serial two-fold dilutions to obtain final concentrations of 2000, 1000, 500, 250, 125, and 62.5 µg/mL. Albendazole, a reference anthelmintic, was used as a positive control at a concentration of 250 µg/mL. DMSO served as the negative control, present at 1.25% in the final test solutions [12].

For the biological assays, adult *Haemonchus contortus* worms were manually harvested using forceps and transferred into Petri dishes. The experimental procedure followed the modified protocol of Hounzangbé-Adoté et al. [13]. Ten live adult worms were placed in Petri dishes containing 3 mL of the 2000 µg/mL plant extract solution. Positive (albendazole at 250 µg/mL) and negative (1.25% DMSO) controls were included in parallel.

Worms were incubated at room temperature (25–27 °C) for 24 hours. During this period, worm motility was monitored under a microscope (×40 magnifications) every six hours. A worm was considered dead if no movement was observed for 60 seconds. To confirm mortality, Petri dishes were

immersed in warm water (45 °C) for 60 seconds under microscopic observation. Worms that failed to exhibit any undulatory movement following stimulation were classified as non-viable.
The mortality rate of adult worms at each extract concentration was calculated using the following formula :

Mortality (%) =

$$\left(\frac{\text{Number of dead worms}}{\text{Number of live adult worms placed in the Petri dishi}}\right) \times 100$$

When a plant extract exhibited adulticidal activity at a concentration of 2000 µg/mL, additional dilutions were prepared to determine the lethal concentration required to induce 95% mortality (LC_{95}) in the *Haemonchus contortus* population. To this end, the same bioassay protocol was applied using extract concentrations of 1000, 500, 250, 125, and 62.5 µg/mL.
Each treatment, including all extract concentrations and controls, was tested in triplicate to ensure experimental reliability.

Statistical analysis

The effect of the plant extracts on *H. contortus* was assessed by calculating mortality rates among adult worms. To compare the mean mortality rates induced by the different extract concentrations, a one-way ANOVA ($\alpha < 0.05$) was conducted. When statistically significant differences were detected, post-hoc comparisons were made using Tukey's test to rank the means. For extracts that demonstrated efficacy at 2000 µg/mL, the LC_{95} value was estimated by performing a linear regression analysis between extract concentrations and observed mortality rates, with a significance threshold set at $p < 0.05$. All statistical analyses were performed using XLSTAT 2017.02 software integrated within Microsoft Excel version 16.4393.

RESULTS

The **Table 1** presents the activity of *Albizia adianthifolia*, *Ficus lutea*, and *Morus mesozygia* extracts on adult *Haemonchus contortus* worms as a function of exposure time. After 6 hours of contact, the mortality rates induced by the three plant extracts remained relatively low (<40%). In contrast, the positive control (albendazole) produced 100% mortality within the same timeframe. A clear time-dependent increase in worm mortality was observed, particularly at higher extract concentrations. At the maximum concentration tested (2000 µg/mL), *A. adianthifolia* induced 30% mortality after 6 hours, reaching complete lethality (100%) after 18 hours. *F. lutea* showed a comparable trend, causing 35% mortality at 6 hours and peaking at 95% mortality after 24 hours. The activity of *M. mesozygia* was comparatively moderate, with a 60% mortality rate observed after 24 hours of exposure at the same concentration. For concentrations below 2000 µg/mL, the most pronounced effect was observed for *A. adianthifolia* at 1000 µg/mL, which induced approximately 85% mortality after 24 hours.Among the three plant extracts tested, *Albizia adianthifolia* and *Ficus lutea* exhibited the highest adulticidal activity against *H. contortus*, especially at extended exposure durations, as illustrated in **Figure 1**.
Figure 2 summarizes the 95% lethal concentrations (LC_{95}) for each plant extract. *A. adianthifolia* yielded the lowest LC_{95} value (1652.89 µg/mL), followed by *F. lutea* (2002.63 µg/mL) and *M. mesozygia* (3876.93 µg/mL). Based on these findings, *Albizia adianthifolia* demonstrated the greatest efficacy against adult *H. contortus* worms.

Table 1 : Mortality percentages of adult *Haemonchus contortus* worms exposed to different concentrations of plant extracts

Plant extracts	Concentrations (µg/mL)	Worm exposure time to extracts in hours				
		0	6	12	18	24
Albizia adianthifolia	62.5	0±0	0 ± 0^{e}	0 ± 0^{h}	25 ± 7.1^{h}	30 ± 0.0^{g}
	125	0±0	0 ± 0^{e}	35 ± 7.1^{ef}	50 ± 0.0^{d}	50 ± 14.1^{e}
	250	0±0	0 ± 0^{e}	35 ± 7.1^{ef}	45 ± 7.1^{e}	55 ± 7.1^{de}
	500	0±0	0 ± 0^{e}	40 ± 14.1^{e}	55 ± 7.1^{d}	60 ± 14.1^{d}
	1000	0±0	10 ± 0.0^{d}	50 ± 0.0^{d}	70 ± 0.0^{c}	85 ± 7.1^{b}
	2000	0±0	30 ± 0.0^{b}	75 ± 7.1^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}
Ficus lutea	62.5	0±0	0 ± 0^{e}	0 ± 0^{h}	30 ± 0.0^{g}	30 ± 0.0^{g}
	125	0±0	0 ± 0^{e}	25 ± 7.1^{g}	35 ± 7.1^{fg}	40 ± 0.0^{f}
	250	0±0	10 ± 0.0^{d}	30 ± 14.1^{f}	40 ± 14.1^{f}	45 ± 7.1^{ef}
	500	0±0	0 ± 0.0^{e}	40 ± 0.0^{e}	70 ± 0.0^{c}	50 ± 14.1^{e}
	1000	0±0	20 ± 0.0^{c}	55 ± 7.1^{cd}	70 ± 0.0^{c}	75 ± 7.1^{c}
	2000	0±0	35 ± 7.1^{b}	65 ± 7.1^{c}	90 ± 14.1^{b}	90 ± 0.0^{b}

Morus mesozygia	62.5	0±0	0±0^{e}	0±0^{h}	0±0^{j}	20±0.0^{h}
	125	0±0	0±0^{e}	0±0^{h}	10±0.0^{i}	25±7.1^{gh}
	250	0±0	0±0^{e}	30±14.1^{f}	35±7.1^{fg}	40±14.1^{f}
	500	0±0	0±0^{e}	30±14.1^{f}	40±14.1^{f}	40±0.0^{f}
	1000	0±0	0±0^{e}	40±0.0^{e}	45±7.1^{e}	45±7.1^{ef}
	2000	0±0	20±0.0^{c}	35±7.1^{ef}	50±0.0^{d}	60±14.1^{d}
Negative control	DMSO (1.25 %)	0±0	0±0^{e}	0±0^{h}	0±0^{j}	10±0.0^{i}
Albendazole (Positive)	250 µg/mL	0±0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}

Mortality rates with distinct letters in the same column are statistically different from each other (P<0.05)

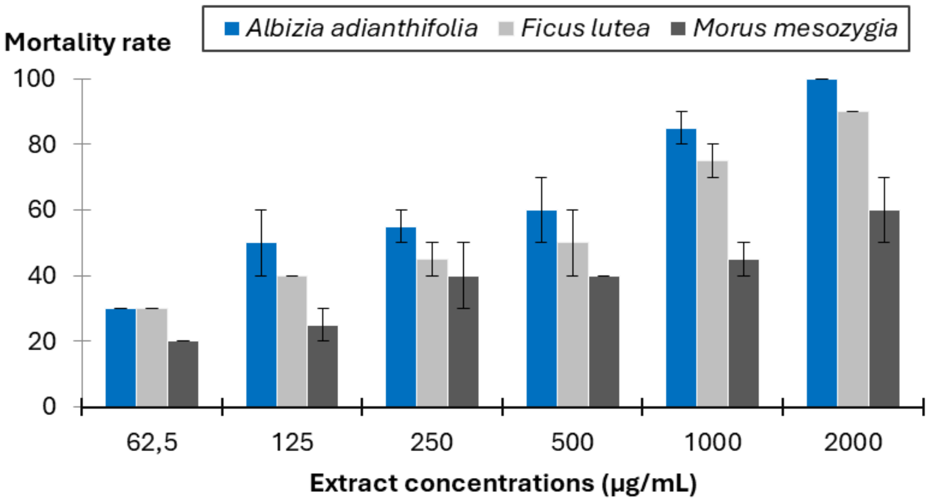

Figure 1 : Mortality rate of adult *Haemonchus contortus* worms induced by *Albizia adianthifolia*, *Ficus lutea* and *Morus mesozygia* after 24 hours of exposure to different concentrations of extracts

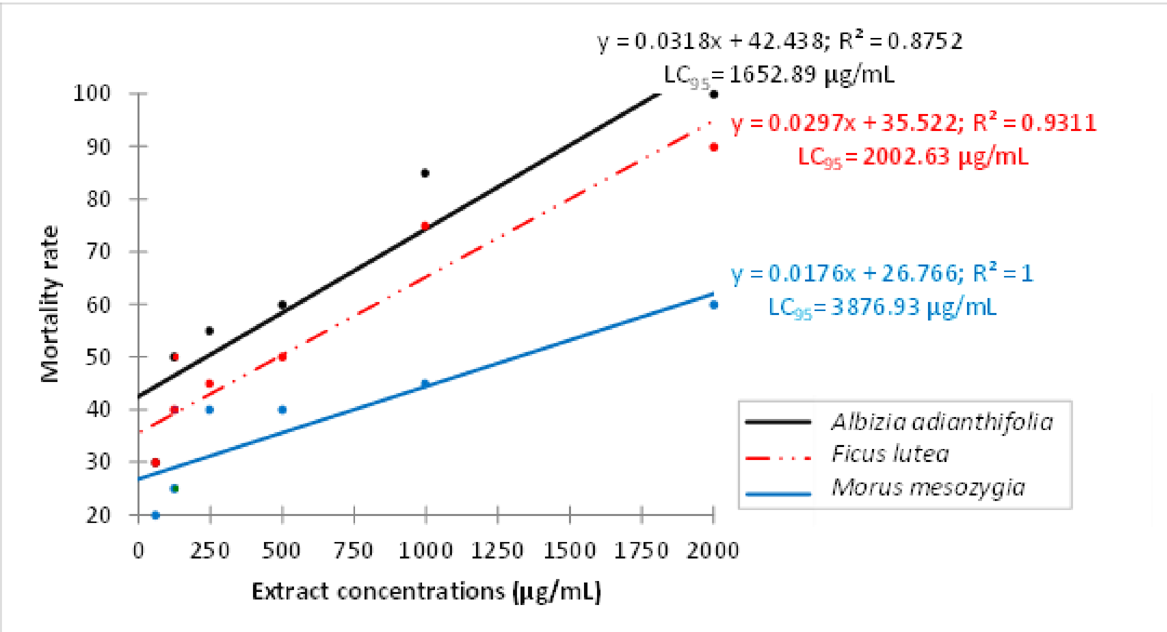

Figure 2 : Lethal concentrations 95% (LC_{95}) of plant extracts on adult *Haemonchus contortus* worms

DISCUSSION

The adulticidal activity of the hydro-methanolic extracts from the three plant species was assessed against adult *Haemonchus contortus*. Among them, *Morus mesozygia* exhibited only moderate efficacy, whereas *Ficus lutea* and *Albizia adianthifolia* demonstrated substantially stronger effects. Notably, the *A. adianthifolia* extract achieved 100 % worm mortality after 24 hours of exposure, with an LC_{95} of 1652.89 µg/mL. These results align with earlier findings showing that the same extracts are anthelmintic against L_1 and L_2 larvae of *H.* contortus [14].

Both *F. lutea* and *A. adianthifolia* belong to forage-plant families (Moraceae and Leguminosae) that include multiple species already reported to possess *in vitro* anthelmintic activity. The Moraceae family encompasses numerous anthelmintically active species [15,16,17,18], and our results further confirm its antiparasitic potential. Likewise, the Leguminosae are recognized for their richness in species active against gastrointestinal nematodes [19,20] Within the Leguminosae, the genus *Albizia* itself contains several bioactive species such as, *Albizia anthelmintica* and *Albizia lebbeck*, with documented efficacy against nematodes, trematodes, and cestodes [21,22,23].

A variety of biochemical classes underpin the anthelmintic activity of plant extracts, including proteinases [24], alkaloids [25], saponins [26], and polyphenols such as condensed tannins and flavonoids [19]. Although several classes are implicated, condensed tannins have been most frequently identified over the past 20 years for activity against gastrointestinal nematodes. Indeed, total phenols, and specifically condensed tannins and flavonoids, were quantified in the hydro-methanolic extracts of *A. adianthifolia* and *F. lutea*, suggesting these metabolites drive the adulticidal effects observed [14]. Moreover, condensed tannins are the principal compounds responsible for the anthelmintic activity of several temperate-legume forages such as, pedunculate (*Lotus pedunculatus*) and horned (*Lotus corniculatus*) birdsfoot trefoil, sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*), and dorycnium (*Dorycnium rectum*) [19,27]. The efficacy of tannins against major parasitic nematodes has been demonstrated both *in vitro* and *in vivo* under experimental infestation of sheep and goats [28,29,30].

In vitro experiments using tannin inhibitors have confirmed the central role of these compounds in anthelmintic activity. Condensed tannins bind to egg and larval membranes, inhibiting hatching and inducing larval death [31]. They also interact with adult-worm surface proteins, disrupting essential physiological functions [32]. *In vivo*, ingestion of tannin-containing forages by sheep and goats reduces fecal egg excretion of *H. contortus*, impairs adult-female worm fertility, and disrupts pasture-infestation cycles by hindering larval development in feces [33].

Taken together, these observations support the hypothesis that the anthelmintic efficacy of the extracts examined here derives principally from condensed tannins, as identified in *Albizia adianthifolia* and *Ficus lutea* [14]. Future work incorporating specific tannin inhibitors will be essential to definitively confirm this mode of action.

CONCLUSION

Pasture parasitism remains an enduring global challenge in livestock production. Integrating forage species endowed with intrinsic anthelmintic properties offers a sustainable approach to gastrointestinal nematode control. *In vitro* evaluations of hydro-methanolic extracts from three forage plants have demonstrated their capacity to reduce worm burden in livestock. Notably, *Albizia adianthifolia* and *Ficus lutea* exhibited potent adulticidal activity against *Haemonchus contortus*, a principal pathogenic nematode in small ruminants. The anthelmintic efficacy of plant extracts is primarily attributed to their repertoire of secondary metabolites. Accordingly, *A. adianthifolia* and *F. lutea* are presumed to contain bioactive compounds, particularly condensed tannins, that underlie their demonstrated nematocidal effects. Future investigations employing specific tannin inhibitors are warranted to delineate the precise role of condensed tannins in mediating these biological activities.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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