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HELMINTHOLOGY - ORIGINAL PAPER

In vitro anthelmintic effects of *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitragyna inermis* leaf extracts on *Haemonchus contortus*, an abomasal nematode of small ruminants

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Abstract

Gastrointestinal nematodes remain a major constraint on the health, welfare, and production of small ruminants. This study was conducted to evaluate three plant extracts (from *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitragyna inermis*) as effective remedies against gastrointestinal parasites of small ruminants. Phytochemical screening was conducted on the plant leaves, and the potential anthelmintic properties of these plants were tested in vitro on *Haemonchus contortus* using the egg hatch, larval migration, and adult worm motility assays. The phytochemical screening of the leaves revealed the presence of several bioactive components in all the plants. The number of eggs that hatched was reduced in a concentration-dependent manner ($p < 0.01$) upon treatment with the methanol extract of *B. ferruginea* and the acetone extracts of *C. glutinosum* and *M. inermis*. The inhibitory effect of the acetone extract of *B. ferruginea* and the methanol extracts of *C. glutinosum* and *M. inermis* was not concentration-dependent ($p > 0.05$). There was a significant difference ($p < 0.05$) in the reduction in larval migration between the lowest concentrations (75 to 150 μg/mL) and the highest concentrations (300 to 1200 μg/mL) of plant extracts. The ability of plant extracts to affect the mobility of the adult worms was not concentration-dependent ($p > 0.05$); however, it was dependent on the time of incubation ($p < 0.01$). At the highest concentration (2400 μg/mL), all adult worms were motionless after 24 h of exposure, while at the lowest concentration (< 150 μg/mL), this occurred after 48 h of exposure. *M. inermis and C. glutinosum* extracts were more effective than *B. ferruginea* extracts ($p < 0.05$). Overall, these results suggest that these plants used by small-scale farmers possess antiparasitic properties useful for helminthiasis control. However, the effects of the plants remain to be confirmed via in vivo assays and toxicity tests in further studies.

Keywords *Bridelia ferruginea* · *Combretum glutinosum* · *Haemonchus contortus* · *Mitragyna inermis* · Anthelmintic activity

Background

Livestock production is one of the main means of achieving improved standards of living in many regions of the developing world. In sub-Saharan African countries, livestock plays a crucial role both in national economies and the livelihood of rural communities (Sibanda and Dube 2014). In Benin, small ruminants can contribute up to 44% of the agricultural gross domestic product (MAEP 2011), providing milk, meat, and wool as well as raw materials for the agri-food industry (Mini et al. 2013). In addition, small ruminant breeding provides an input for crop production and soil fertility in the form of manure (Mini et al. 2013). Gastrointestinal parasitic infections can cause economic losses and have serious and detrimental impacts on the livelihoods of small farmers in the developing world, where climate change, poor sanitation, poverty, and demographic conditions favor the development and

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transmission of helminth parasites. Among the helminths identified in Benin, the parasites of the genus *Haemonchus* represent the dominant species in cattle and small ruminants (Salifou 2006; Hounzangbé-Adoté et al. 2005). Diseases caused by *Haemonchus contortus* have numerous negative impacts on the productivity and fertility of herds, such as losses due to mortality and morbidity, weight loss, depressed growth, poor fertility, and decreased physical power (Stein et al. 2011).

Over the last few decades, the main way of controlling this parasite has been through the use of chemical anthelmintics. However, these treatments are no longer effective, as the worm populations have developed resistance to these chemicals (Hoste et al. 2006). In addition, synthetic drugs are rarely used by rural communities due to their high costs, unavailability, and inaccessibility (Hammond et al. 1997). Consequently, the need to find alternative solutions is becoming urgent. Therefore, the use of natural local resources such as *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitragyna inermis*, which are less expensive and more efficient than synthetic drugs and have low toxicity, remains the most relevant alternative to modern anthelmintic drugs (Maphosa et al. 2010; Alowanou et al. 2015).

Different parts of *B. ferruginea* (Koné and Atindehou 2007; Djoueché et al. 2011), *C. glutinosum* (Kaboré et al. 2007; Djoueché et al. 2011), and *M. inermis* (Koné and Atindehou 2007; Kaboré et al. 2007; Attindéhou et al. 2012) have been reported to be useful in traditional medicine against gastrointestinal disorders and helminth infections in humans and livestock in West and Central Africa. Therefore, there is a vital need for scientific validation of the traditional use of these plants.

A previous study has reported that the stem bark extract and chemical contents of *B. ferruginea* showed a significant antiparasitic effect on the larval development of *H. contortus* (Koné et al. 2005). Recently, the stem bark extract and chemical contents of *B. ferruginea* leaves demonstrated significant anthelmintic activity against several parasitic worms, especially *Fasciola gigantica* (liver fluke), *Taenia solium* (tapeworm), and *Pheretima posthuma* (earthworm, annelid) (Lasisi and Kareem 2011). Bark extracts of *M. inermis* were investigated in vitro for their larvicidal activity against infective larvae of *H. contortus* and yielded satisfactory findings (Diehl et al. 2004). However, the effects of *B. ferruginea* and *M. inermis* on egg hatching and adult worm motility, as well as the effects of *C. glutinosum* on the three life cycle stages of parasitic nematodes, are still unknown. Moreover, all these studies evaluated the effect of the bark extracts instead of the leaf extracts, which are used in the present work. Many attempts have been made to study the anthelmintic potential of the three plants, but further efforts are needed.

Therefore, the current study was conducted to explore the potential anthelmintic properties of the leaves of these three tropical plants on *H. contortus* from Western and Central Africa. The plants were chosen on the basis of a survey conducted on the prevalence and frequency of use of these plants by small-scale farmers in the Republic of Benin against parasitic infections in ruminants (Alissou 2013).

Methods

Collection and preparation of plant material

The leaves of *B. ferruginea* Benth. (Euphorbiaceae), *C. glutinosum* Perr. Ex. D.C. (Combretaceae), and *M. inermis* (Willd.) O. Ktze., (Rubiaceae) plants were collected in December 2015 from Abomey-Calavi (South Benin), Kandi (North Benin), and Comé (South West Benin). Specimens were identified at the national herbarium of Benin. The leaf samples of each plant, harvested from their natural habitat, were dried indoors at room temperature for 9 days. Then, the dried leaves were transformed into powder using a laboratory grinder (Bender and Hobein 8042 Zurich) and kept at room temperature until use.

Plant extract preparation

The leaf powder (50 g) was mixed in 500 mL of solvent for 2 h at 40 °C with the aid of a magnetic stirrer (IKA C-MAG HS7). Two types of solvent were used: a mixture of methanol and distilled water and a mixture of acetone and distilled water, each in a 70:30 ratio. The solution was subsequently filtered through filter paper, and the resulting filtrate was evaporated off using a Rotavapor (BUCHI RII) at 47 °C to remove the solvents completely. The resulting extracts were lyophilized, and the yield of extracts from each plant was calculated. The extracts obtained were stored at 4 °C and used for phytochemical and biological assays.

Phytochemical analysis

Qualitative phytochemical screening of plant leaves was carried out using the standard precipitation and coloration reactions as described by Houghton and Raman (1998). Other secondary metabolites were assayed by different methods (Houghton and Raman 1998).

Procedures for biological assays

The effects of the plant extracts on the three main stages of the parasite life cycle, i.e., the eggs, second-stage larvae (L3s), and adult worms, were measured using different laboratory procedures, namely, egg hatching assays, inhibition of larval migration, and adult motility inhibition (Hounzangbé-Adoté et al. 2005).

Egg hatch assay

A modified version of the method of Coles et al. (1992) was used to assess anthelmintic resistance. Parasite eggs were obtained from donor sheep experimentally infected with *H. contortus*. The eggs were extracted using a salt flotation step, washed repeatedly with PBS (phosphate-buffered saline; pH 7.2, 0.15 M), and distributed in 96 multiwell plates at a concentration of 100 eggs/well. One hundred microliters of each plant extract was prepared with PBS to achieve different concentrations (75, 150, 300, 600, 1200, and 2400 μg/mL), which were subsequently added to separate wells containing eggs. Wells containing PBS (negative control) and thiabendazole at 500 μg/mL in PBS (positive control) were also included on the assay plate. The assay plate was then incubated at 27 °C for 48 h. The eggs that hatched and those that failed to hatch were counted under a microscope. The test was replicated five times for each plant extract concentration. The inhibition percentage of hatching for each concentration was evaluated using a modification of the formula of Coles et al. (1992).

Larval migration inhibition assay

The larval migration inhibition assay, (LMIA) was conducted as described by Rabel et al. (1994) to measure inhibitory activity against infective larvae (L3s), which were obtained following fecal culture from sheep previously infected experimentally with *H. contortus* strains. Feces were collected and cultured at room temperature for 10 days. The L3s were then extracted from the fecal mass using a Baermann apparatus. Infective L3s were incubated for 3 h at 20 °C in PBS solutions of plant extracts at the concentrations of 75, 150, 300, 600, and 1200 μg/mL. The L3s were then washed three times in PBS and centrifuged. After the last washing, 800 μL of L3s at a concentration of 1000 L3s/mL was pipetted onto a 20-μm mesh. The sieve was inserted into a conical tube to touch the surface of the PBS contained therein. Four replicates were run at room temperature for each concentration of plant extracts. In addition, a negative control (L3s incubated in PBS) and a positive control (L3s incubated in levamisole at a concentration of 250 μg/mL) were run in parallel. After 3 h, the L3s above the sieve were discarded, and those that actively migrated through the mesh into the PBS below were counted under the × 10 objective of a microscope. The percentage of LMI was calculated as follows:

$$\mathrm{LMI} = \frac{T-M}{T} \times 100$$

where T is the total number of L3s deposited in the sieve and M is the number of L3s having migrated through the mesh into the PBS.

Adult worm motility inhibition assay

The purpose of this assay was to test the anthelmintic effect of the three plant extracts on adult worm motility. This test was performed according to the method of Hounzangbé-Adoté et al. (2005). Adult worms were obtained from sheep that were experimentally infected with a pure strain of *H. contortus*. Four weeks after infection, the sheep were euthanized. Immediately after death, the abomasum was collected, opened, briefly washed with saline, and placed in a Baermann apparatus with saline solution at 37 °C. After 2 h, the worms that migrated into the saline solution were collected and quickly placed in a 48 multiwell plates at a concentration of three worms per well. The worms were first washed for approximately 1 h in PBS with penicillin and streptomycin at a concentration of 4%. Thereafter, 1-mL volumes of the different concentrations of plant extracts (75, 150, 300, 600, 1200, and 2400 μg/mL) were added to the respective wells. A positive control (levamisole) and a negative control (PBS plus antibiotic) were included on each plate. For each treatment, the measurements were performed using six replicates per concentration per plate. The supernatant was changed every 24 h. The mobility of the adult worms was noted by careful observation under the × 10 objective of a microscope at intervals (6, 12, 18, 24, 30, 36, 42, and 48 h). At each observation time, a motility index was calculated as the ratio of the total number of motionless worms to the total number of worms, referring in each case to the overall number of worms present in the six replicates.

Statistical analysis

For assays of egg hatching and larval migration, significant mean differences between treatments in the proportions of unhatched eggs and the migration inhibition migration rates were assessed by two-way analysis of variance using a general linear model (GLM) procedures through the MASS package (Venables and Ripley 2002) in the software R (R Core Team 2013). The comparison of the three plants for each concentration in both assays consisted of a Tukey-Kramer HSD test performed with the agricolae package in R (R Core Team 2013). In adult worm assay, for each treatment, the number of motionless worms was recorded at each measurement time, and the survival data were assessed using Student's t test in R (R Core Team 2013). The concentration of each extract or positive control required to inhibit 50% of egg hatching, larval migration, or adult worms' motility (IC50) was computed, along with its 95% confidence intervals (CI), using the logarithmic nonlinear regression function in R software (R Core Team 2013).

Table 1 Extraction yields of the three plant species

Plants	Solvents	Weight of plant extracts (g)			Yield of extraction (%)
		Repetition 1	Repetition 2	Repetition 3	
B. ferruginea	Acetone	13.5	11.9	12.3	25.13
	Methanol	10.9	08.7	09.4	19.33
C. glutinosum	Acetone	10.0	09.5	10.6	20.06
	Methanol	06.1	5.3	6.5	11.93
M. inermis	Acetone	10.1	10.7	12.7	22.4
	Methanol	10.3	9.8	11.2	20.86

B. ferruginea, *Bridelia ferruginea*; *C. glutinosum*, *Combretum glutinosum*; *M. inermis*, *Mitragyna inermis*

Results

Extraction yield

The acetone extracted more material than the methanol irrespective of the plant used for extraction (Table 1). The highest extract yield was obtained from *B. ferruginea* using acetone, and the lowest was obtained from *C. glutinosum* with methanol (Table 1).

Phytochemical analysis

The phytochemical screening of the leaf extracts of *B. ferruginea*, *C. glutinosum*, and *M. inermis* revealed the presence of several bioactive components, such as tannins (condensed and gallic), flavonoids, saponins, alkaloids, and reducing compounds (Table 2). Anthraquinones, terpenes, and glycosides were detected in the extract of *M. inermis*, but this was not the case for *C. glutinosum*. Moreover, terpenes were not detected in the extract of *B. ferruginea* (Table 2).

Table 2 Secondary metabolites recovered from extracts of three plants, namely, *B. ferruginea*, *C. glutinosum*, and *M. inermis*

Metabolites	Medicinal plants screened		
	B. ferruginea	*C. glutinosum*	*M. inermis*
Condensed tannins	+	+	+
Gallic tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Glycosides	+	−	+
Anthraquinones	+	−	+
Terpenes	−	−	+
Reducing compounds	+	+	+

The presence and absence of secondary metabolites are denoted by "+" and "−", respectively

Effects on egg hatching

The positive control and plant extracts significantly reduced *H. contortus* egg hatching in vitro ($p < 0.001$). Regarding the three plant extracts, the reduction in egg hatching varied from 13.6 to 77.2% as the eggs were exposed to increasing concentrations of the plant extracts (Fig. 1). The methanol extract of *B. ferruginea* and the acetone extracts of *C. glutinosum* and *M. inermis* showed significant ($p < 0.01$) concentration-dependent effects on egg hatching. However, with the acetone extract of *B. ferruginea* and the methanol extracts of *C. glutinosum* and *M. inermis*, the inhibitory effect was not concentration-dependent ($p > 0.05$) (Fig. 1). The extracts of *B. ferruginea*, *C. glutinosum*, and *M. inermis* reduced egg hatching from 17.11 to 58.10%, 13.6 to 67.56%, and 20.27 to 77.02%, respectively, compared with the negative control (Fig. 1). The extracts of *M. inermis* and *C. glutinosum* on egg hatching inhibition were more effective than those of *B. ferruginea* ($p < 0.05$). Moreover, the concentrations of the extract required to inhibit 50% of eggs (IC50) from hatching, known as the 50% lethal concentration (LC50), were 59.14 and 63.83 μg/mL for *M. inermis* and *C. glutinosum*, respectively, whereas that of *B. ferruginea* was 87.41 μg/mL (Table 4).

Effects on infective larvae

All the tested plant extracts except the acetone extract of *M. inermis* significantly reduced ($p < 0.05$) the larval migration of *H. contortus* in a non-concentration-dependent manner, with inhibition ranging from 17.36 to 67.52%, 27.65 to 68.27%, and 21.22 to 61.74% for *B. ferruginea*, *C. glutinosum*, and *M. inermis*, respectively (Fig. 2). The acetone extract of *M. inermis* demonstrated a significant ($p < 0.05$) concentration-dependent effect, with inhibition ranging from 21.54 to 61.09%, compared with the negative control. Levamisole, used as a positive control, exhibited a more significant effect ($p < 0.01$) with 92.6% larval migration inhibition (Fig. 2). A significant effect ($p < 0.05$) on larval migration was observed from

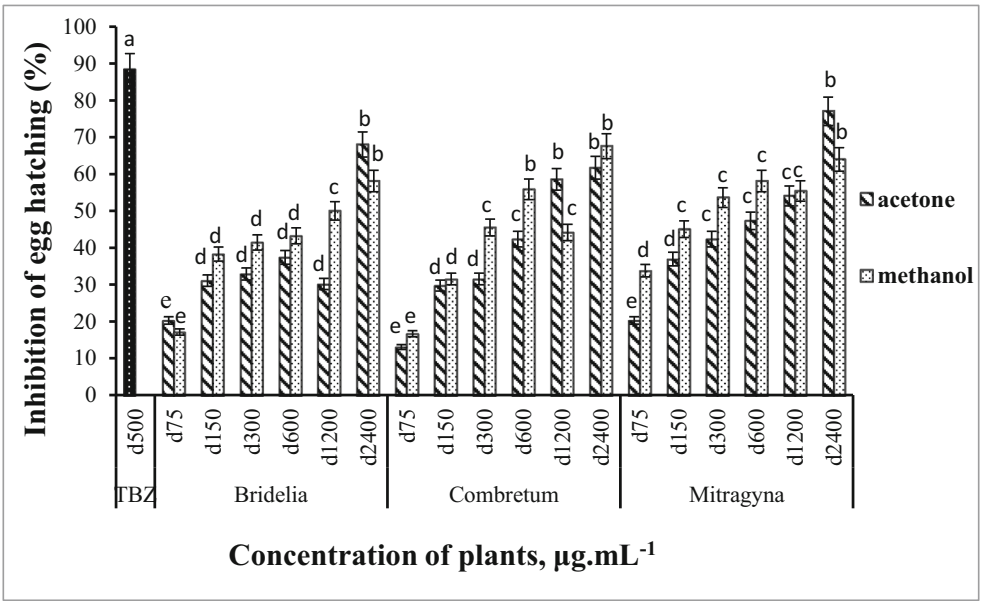

Fig. 1 Effects of the three plant extracts at concentrations of 75, 150, 300, 600, 1200, and 2400 µg/mL and the positive control, thiabendazole (500 µg/mL), on the hatching of *H. contortus* eggs. Each bar of the chart represents the mean ± SEM of the number of eggs that failed to hatch. The letters on each bar compare the results of the different plant extracts according to Tukey's HSD test. Different letters indicate a significant difference between values at $p < 0.05$. TBZ, thiabendazole; d500, 500 µg/mL; d75, 75 µg/mL; d150, 150 µg/mL; d300, 300 µg/mL; d600, 600 µg/mL; d1200, 1200 µg/mL; d2400, 2400 µg/mL; Mitragyna, *Mitragyna inermis*; Combretum, *Combretum glutinosum*; Bridelia, *Bridelia ferruginea*; SEM, standard error of the mean

moderate (300 µg/mL) to higher (1200 and 600 µg/mL) concentrations of each plant extract (Fig. 2). Moreover, the concentrations of the extract required to inhibit 50% of larvae (IC50) from migration, known as the 50% lethal concentration (LC50), were 96.62, 97.84, and 97.51 µg/mL^{-1} for *M. inermis*, *B. ferruginea*, and *C. glutinosum*, respectively (Table 4).

Effects on adult worm motility

The positive control and plant extracts significantly ($p < 0.001$) reduced the motility of adult *H. contortus* worms in vitro compared with the negative control (PBS). This reduction was not concentration-dependent ($p > 0.05$) but was dependent on incubation time

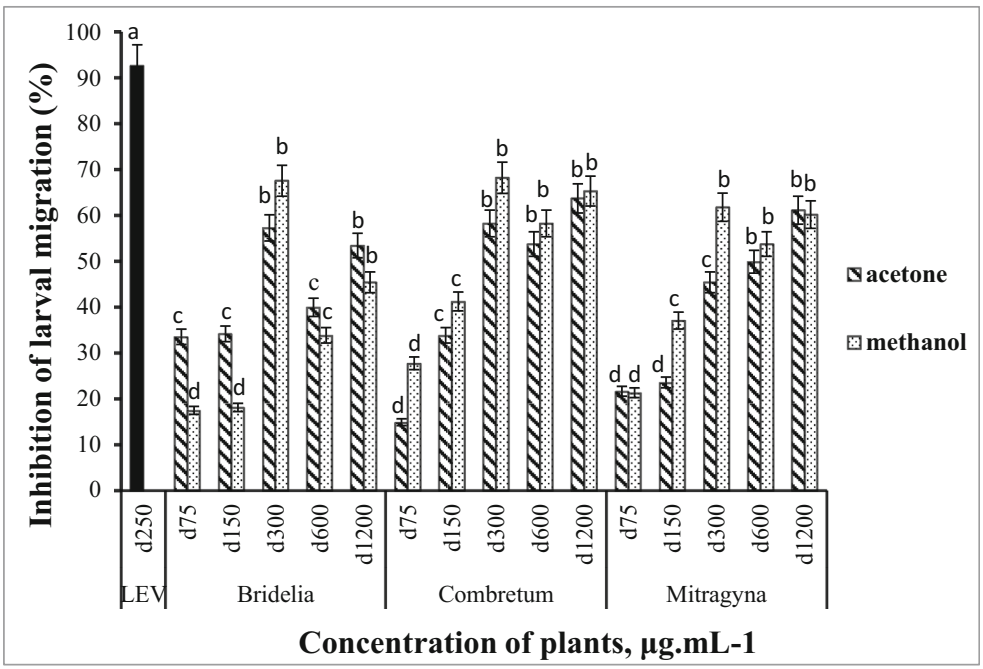

Fig. 2 Effects of the three plant extracts at concentrations of 75, 150, 300, 600, and 1200 µg/mL and the positive control, levamisole (250 µg/mL), on *H. contortus* larval migration. Each bar of the chart represents the mean ± SEM of the number of larvae not migrated. The letters on each bar compare the results of different plant extracts based on Tukey's HSD test. Different letters indicate a significant difference between values at $p < 0.05$. LEV, levamisole; d250, 250 µg/mL; d75, 75 µg/mL; d150, 150 µg/mL; d300, 300 µg/mL; d600, 600 µg/mL; d1200, 1200 µg/mL; Mitragyna, *Mitragyna inermis*; Combretum, *Combretum glutinosum*; Bridelia, *Bridelia ferruginea*; SEM, standard error of the mean

Table 3 Effects of various concentrations of extracts (75, 150, 300, 600, 1200, and 2400 μg/mL) from the three plants on the motility of adult *Haemonchus contortus* worms over time, assessed by the adult worm motility assay

		% of motile worms		
	Time (h)	24	36	48
	Concentration (μg/mL)			
PBS		75	25	0
LEV	500	0	0	0
	250	0	0	0
	125	0	0	0
Water/acetone extract	2400	25	0	0
B. ferruginea	1200	12.5	0	0
	600	12.5	0	0
	300	25	0	0
	150	50	12.5	0
	75	50	12.5	0
Water/methanol extract	2400	25	0	0
B. ferruginea	1200	12.5	0	0
	600	12.5	0	0
	300	25	0	0
	150	50	12.5	0
	75	50	25	0
Water/acetone extract	2400	0	0	0
C. glutinosum	1200	12.5	0	0
	600	12.5	0	0
	300	12.5	0	0
	150	37.5	0	0
	75	50	12.5	0
Water/methanol extract	2400	0	0	0
C. glutinosum	1200	12.5	0	0
	600	37.5	0	0
	300	12.5	0	0
	150	37.5	12.5	0
	75	62.5	12.5	0
Water/acetone extract	2400	0	0	0
M. inermis	1200	12.5	0	0
	600	12.5	0	0
	300	12.5	0	0
	150	37.5	0	0
	75	50	12.5	0
Water/methanol extract	2400	0	0	0
M. inermis	1200	25	0	0
	600	12.5	0	0
	300	12.5	0	0
	150	37.5	0	0
	75	50	12.5	0

($p < 0.01$) (Table 3). In addition, the concentrations of extracts of *M. inermis*, *B. ferruginea*, and *C. glutinosum* required to inhibit 50% of worms (IC50) from motility were 131.34, 166.76, and 136.25 μg/mL, respectively (Table 4).

After 24 h of exposure, total inhibition was observed in the wells treated with the positive control and the highest concentration (2400 μg/mL) of *M. inermis* and *C. glutinosum*, while the negative control treatment left 75% of worms alive. At the same time, the worms' motility was inhibited 87.5% with plant extracts of 1200, 600, and 300 μg/mL and at least 50% with lower concentrations (150 and 75 μg/mL) (Table 3).

High inhibition of adult *H. contortus* worms' motility was observed following exposure to each plant extract, irrespective of the concentration, after 36 h (Table 2). The reduction in motility was 100% for all plant extracts and concentrations with the exception of the two lowest concentrations (75 and 150 μg/mL) and the negative control, where the percentage values of motile worms were 12.5% (both low concentrations) and 25%, respectively (Table 3).

Following 48 h of exposure to different concentrations of plant extracts or the control treatments, there was no evidence of motility of *H. contortus*. Extracts of *C. glutinosum* and *M. inermis* had significant and rapid effects on the survival of adult worms at higher concentrations. However, low concentrations of the three plants were also effective over a longer period of time (Table 3).

These effects were expressed as the percentage of immobile worms compared with the total number in the wells, depending on time and concentrations of extracts or control treatments.

Discussion

For in vitro studies, *H. contortus* proved to be a good test worm because of its long survival in PBS. Thus, this worm and some other worms such as *Strongyloides* have previously been used for in vitro studies by some researchers and have yielded satisfactory outcomes (Hounzangbé-Adoté et al. 2005; Azando et al. 2011; Dédéhou et al. 2014). Different trials were performed to investigate the capacity of acetone and methanol extracts of *B. ferruginea*, *M. inermis*, and *C. glutinosum* leaves to disrupt the life cycle of *H. contortus*. The extraction yield obtained from the three plants did not influence their efficacy because the most effective extracts, i.e., the acetone extracts of *M. inermis* and *C. glutinosum*, had the lowest yield. Moreover, those two plants displayed the lowest concentrations of extract required to inhibit 50% (IC50) of egg hatching and adult worm motility in the present study. Overall, concentrations of plant extracts between 75 and 2400 μg/mL^{-1} showed significant anthelmintic activity against egg hatching, larval migration, and adult worm motility with a great degree of variation.

Table 4 Extract concentration required to inhibit 50% of effect (IC50) in various anthelminthic assays

Assay	Treatment	IC50 (μg/mL)	CI (95%) Lower (μg/mL)	Upper (μg/mL)	R^2
EHA	Thiabendazole	17.25	9.28	32.02	0.99
	M. inermis	59.14	31.84	109.8	0.91
	B. ferruginea	87.41	47.07	162.29	0.85
	C. glutinosum	63.83	25.14	162.01	0.93
LMIA	Levamisole	18.55	9.93	34.63	0.98
	M. inermis	96.62	51.76	180.39	0.94
	B. ferruginea	97.84	52.41	182.67	0.91
	C. glutinosum	97.51	52.23	182.06	0.93
AMIA	*M. inermis*	131.34	70.36	145.22	0.98
	B. ferruginea	166.76	89.33	311.35	0.86
	C. glutinosum	136.25	72.99	254.39	0.89

The concentrations of the extracts or positive control required to inhibit 50% of egg hatching, larval migration, or adult worms' motility (IC50) were generated, along with their 95% confidence intervals (CIs), by the logarithmic nonlinear regression function of the software R

Concentration-dependent effects on egg hatching were found with the methanol extract of *B. ferruginea* and the acetone extracts of *C. glutinosum* and *M. inermis*, whereas no concentration-dependent effects on egg hatching were obtained with the acetone extract of *B. ferruginea* or the methanol extracts of *C. glutinosum* and *M. inermis*. The findings obtained from the egg hatching assay indicated that the three plant extracts had in vitro ovicidal activity. The observed ovicidal activity of plant extracts could be because the active compounds penetrate the eggshell and stop the segmentation of the blastomeres or paralyze the larvae inside embryonated eggs (Wabo-Poné et al. 2011). In a recent review, Alowanou et al. (2015) reported that the leaves of the three tested plants contain many active compounds such as tannins, flavonoids, polyphenol, coumarins, and alkaloids that are assumed from the literature to be responsible for their anthelmintic activity. Moreover, the leaves of the three plants are widely consumed by ruminants; therefore, these plants, when used as herbal drugs or combined with food supplements, can help to modulate helminthiasis through long-term treatment of animals on a given farm. These treatments may reduce the hatchability of the eggs excreted in the feces, resulting in both a reduced risk of reinfection and lightened worm loads by decreasing pasture contamination (Zangueu et al. 2018).

The in vitro migration assay is a rapid and effective tool to quantify the paralytic effects of drugs on nematodes (D'Assonville et al. 1996). The effects of plant extracts on the third-stage larvae, as measured by the larval migration inhibition assay, were not concentration-dependent except in the case of the acetone extract of *M. inermis*. Moreover, a reduction in larval migration by more than 50% was observed with the three plant extracts, even at the concentration of 300 g/mL. The larvicidal effect of *M. inermis* and *B. ferruginea* observed in the present study has been reported in previous studies conducted on the bark extract of *M. inermis* by Diehl et al. (2004) and the stem bark extract of *B. ferruginea* by Koné et al. (2005) on the same parasite. However, the extracts of *M. inermis* and those of *B. ferruginea* showed a larvicidal effect with IC50 values of 96.62 and 97.51 μg/mL, which appears moderate compared with the results reported by Diehl et al. (2004) and Koné et al. (2005), which were 0.849–1.7 mg/mL for *M. inermis* and 0.849 mg/mL for *B. ferruginea*, respectively. The importance of the observed effect could be due to the nature of the test used, since the mechanism involved in the larval development assay is not exactly the same as that of the larval migration assay. Moreover, the various parts of the plant (leaf versus stem bark and bark) and the type of solvent (methanol or acetone-water vs ethanol) used for the extraction were different. Thus, the secondary metabolites present in the plant extract (regarding both quality and quantity) could differ from one extract to another. The ability of acetone and methanol to extract compounds of a wide polarity range at a high yield is greater than that of ethanol or water (Zangueu et al. 2018). Moreover, acetone and methanol were selected in the present study as suitable extractants due to their ability to extract compounds of a wide polarity range, which makes them more suitable than ethanol or water as a solvent for plant secondary compounds (Eloff 1998; Adamu et al. 2013; Mengistu et al. 2017). The mechanisms involved in the effect of plant extracts on infective larvae are not well known. The principle of the larval migration inhibition assay (LMIA) depends on an active migration process through the sieve; therefore, the reduction in larval migration could be due either to larval mortality or to larval paralysis. The larvicidal activity of the different extracts observed in this study may be attributable to the presence of secondary metabolites such as saponins, alkaloids, tannins, and flavonoids found in the leaves of plants. It has been

reported that phenolic compounds including alkaloids, condensed tannins, and flavonoids have been implicated in forms of pharmacological activity such as anthelmintic activity (Makkar et al. 2007). These secondary metabolites may be diffused through the cuticle of larvae or into intestinal cells to inhibit larval migration or cause mortality. Moreover, tannins are able to bind to the free proteins available in the rumen for larval nutrition, and reduced nutrient availability could lead to larval starvation and death (Ademola et al. 2005).

In the adult worm motility inhibition assay (AMIA), the plant extracts were associated with reduced worm motility after 24 h of exposure compared with the negative control. Among the plants tested, *M. inermis* and *C. glutinosum* seemed to yield the most active anthelmintics against adult worms, with 100% motionless worms after 24 h at the maximum concentration of 2400 μg/mL. Similar results have been reported for adult *H. contortus* (Hounzangbé-Adoté et al. 2005) with alcoholic extracts of four tropical plants (*Morinda lucida*, *Carica papaya*, *Newbouldia laevis*, and *Zanthoxylum zanthoxyloides*). In that study, high rates of immobility were recorded after 6 h of exposure to *N. laevis* and after 24 h of exposure to the other species. Motility reduction or complete immobility is an indicator of paralysis or mortality of the worms and constitutes evidence of the effect of the plant extract. The high activity of plant extracts on adult *H. contortus* worms could also be due to different secondary metabolites, as identified in the leaves of plants. These compounds contained in the leaves may create unfavorable conditions for the survival of *H. contortus* adult worms (Azando et al. 2011). In addition, nematode muscles are known to contain excitatory neuromuscular junctions with various receptors for the neurotransmitter acetylcholine (Neal 2002). Some of these metabolites, acting as ganglion stimuli, could activate these neuromuscular junctions and cause spastic paralysis in the worms, leading to their death.

In vitro methods provide some means to screen rapidly for potential anthelmintic activities of the different plant extracts and to analyze the possible mechanisms involved in the interactions between active compounds and parasites. However, due to the divergence in conditions we encountered in vivo and in vitro (availability and/or concentration of any active compounds, metabolic transformation), the results remain merely suggestive and need to be confirmed through in vivo studies with experimental nematode infections in target host species.

Conclusion

The leaf extracts of *B. ferruginea*, *C. glutinosum*, and *M. inermis* showed anthelmintic activity in vitro against egg hatching, larval migration, and adult motility in *H. contortus*. The use of acetone and methanol extracts of the leaves of these three plants may therefore be useful for the control of gastrointestinal nematodes in livestock production, particularly *H. contortus*. Further research is needed to identify, characterize, and better understand the nature of the secondary metabolites that exert the anthelmintic effect and to analyze their mode of action on the nematodes.

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Compliance with ethical standards

Ethics approval and consent to participate The present study was approved and conducted in accordance with the guidelines of the Ethical Committee of University of Abomey – Calavi (EC approval 2015/1134), and after receiving approval, all experiments were conducted using the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP). In addition, it is important to note that consent was obtained from all participants, especially from the animal owners of South and North Benin, who usually use the three plants to treat their animals against parasites of the ruminant gastrointestinal tract.

Consent to publish Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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