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Research article

UPLC-QToF-ESI-MS identification and anthelmintic activity of *Mitragyna inermis* (Willd.) Kuntze (Rubiaceae)

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ABSTRACT

Medicinal plants attract the attention of many researchers to find natural and safe remedies for various resistant diseases. Leaves of *Mitragyna inermis* are widely used in traditional veterinary medicine for the treatment of gastrointestinal strongyles of small ruminants. The aim of the current study is to estimate the antioxidant, anthelmintic and the larval toxicity of the aqueous and hydroethanolic extracts of this plant in addition to the hexane, dichloromethane and ethanol fractions of the hydroethanolic extract. Investigation of the most active extract using Ultra Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Electrospray Ionization Mass Spectrometry (UPLC-QToF-ESI-MS). Both plant extracts showed good antioxidant activity by scavenging the 2,2′-diphényl-1-picrylhydrazyl (DPPH) radical and reducing the ferric ion. Similarly, they were no-toxic to *Artemia salina* larvae (CL_{50} > 0.1 μg/mL). Also, they significantly reduced larval migration and motility of *Haemonchus contortus* adult worms ($p < 0.001$). The hexane, dichloromethane and ethanolic fractions of the hydroethanolic extract showed low activity compared to crude extracts except for the hexane fraction on *H. contortus* adult worms ($p < 0.001$) while it showed a poor result on larvae. It thus appears that the anthelmintic activity of the extract may be linked to the synergistic action of these compounds. The UPLC-QToF-ESI-MS analysis revealed the tentative identification of 15 compounds including 7 alkaloids. The results of the present study confirm the anthelmintic activity of M. inermis in traditional veterinary medicine.

Abbreviations: IC_{50}, concentration at which Fifty percent of DPPH radical were trapped; EC_{50}, 50% effective concentration; ESI, Electrospray Ionization; HPLC, High Performance Liquid Chromatography; HRESI, High Resolution Electrospray Ionization; LC, Liquid Chromatography; MS, Mass Spectrometry; QToF, Quadrupole Time-of-Flight; UPLC, Ultra Performance Liquid Chromatography; DAD, Diode Array Detector; DPPH, 2,2′-diphényl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power.

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1. Introduction

The easy access to medicinal plants and the knowledge of their virtues offer a great opportunity for their uses. In Africa, more than 90% of the population use traditional medicine to solve health problems [1]. In Benin, Dedehou et al. [2] reported that, there are no villages or hamlets, without traditional healers. Not only therapeutic properties benefit people, but also, they are useful for animals, and Benzeggouta [3], estimates that healing with plants is an instinct found in the animals behavior. Thus, to cope with gastrointestinal parasites, especially heamonchosis, which is the main cause of morbidity and mortality in small ruminants and thus creates a shortfall for small breeders [4], the breeders, after several failures to use synthetic drugs, in the search for a reliable, effective and sustainable solution have turned to endogenous knowledge, including medicinal plants. Indeed, synthetic anthelmintics are very expensive for farmers with low income and are not even accessible everywhere and all the time this is due to the lack of veterinary pharmacies in rural areas with high sheep production in Benin [4]. Added to these problems is the resistance developed by parasites in the face of the most commonly used synthetic anthelmintics [5]. Therefore, the use of medical plants potentially endowed with anthelmintic properties remains an asset for breeders. This is the case of *M. inermis*, whose aqueous decoction of leaves is used orally as a remedy in traditional veterinary medicine to treat livestock helminthiasis of ruminants including sheep, goats and cattle [[6–8]]. Although *in vitro* studies have been conducted with acetone and methanolic extracts of this plant [[9,10]] and *in vivo* studies on the powder of these leaves [[11]] with the aim of evaluating these anthelmintic properties, to our knowledge, no study has been able to address the extracts commonly used in traditional medicine for the identification of compounds. Scientific studies to gain a good understanding of the effective dose and the toxicity of these remedies will be necessary for the valorization of this plant in traditional veterinary medicine. With the aim of manufacturing long-term veterinary phytomedicines, responsible for antiparasitic properties, the present study, by bioassay-guided approach was undertaken to further identify constituents, which may contribute to the anthelminthic activity of *M. inermis*.

2. Materials and methods

2.1. Plant material

M. inermis leaves were collected in Zogbodomey in the south of Benin. The plant species was identified and authenticated at the National Herbarium of the University of Abomey-Calavi under the number AA 6713/HNB.

2.2. Extraction, fractionation and isolation

The leaves are dried in a dark place for about two weeks and then ground to a powder using a grinder. The powder is used to prepare the various extracts.

2.2.1. Aqueous extract

Powdered leaves of *M. inermis* (100 g) was suspended in 1 L of distilled water and boiled for 30 min. After decantation and filtration, the filtrate was evaporated under vacuum using a rotary evaporator connected to a pump at a temperature between 50 and 55 °C to give (12.06 g) of the aqueous extract.

2.2.2. Hydroethanolic extract

One hundred gram of air dried *M. inermis* powdered leaves were extracted with ethanol (70%, 1 L) at room temperature for 72 h till complete exhaustion. The extract was evaporated under vacuum to yield (18.06 g) of total hydroethanolic extract. The extract was successively partitioned using solvents of increasing polarity, which allowed to obtain three fractions: the hexane fraction, the dichloromethane fraction, and the ethanolic fraction which is only the hydroethanolic extract exhausted by hexane and dichloromethane. These three fractions were subjected to antiparasitic activity and showed that the anthelmintic power would be linked to the synergistic effect of the compounds.

2.3. Phytochemical screening

The phytochemical screening was carried out on *M. inermis* leaf extract following the method used by Toklo et al. [12,12]. This is a qualitative chemical analysis to search for the major families of secondary metabolites, based on differential precipitation and staining reactions.

2.4. Qualitative analysis of the constituents of the hydroethanolic extract by UPLC-QToF-ESI-MS

2.4.1. Sample preparation

The EtOH/H_2O (7:3) extract was dissolved in HPLC grade methanol in a concentration of 5 mg/mL then filtrated through a syringe-filter-membrane. Aliquots of 5.00 µl were injected into the UPLC–DAD/MS Dionex Ultimate 3000 HPLC (Dionex Softron GmbH Germany), used for performing the analyses.

2.4.2. UPLC-MS conditions

High resolution mass spectra were obtained with a spectrometer QToF Bruker equipped with a HRESI source and provided by SiChem GmbH, Bremen, Germany. The spectrometer operates in positive mode (mass range: 100–1500 Dalton (Da), with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.40 ppm deviation using natrium formate as a calibrant. The following parameters were used for the experiments: sputtering voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 HPLC system consisting of an LC pump, a diode array detector (DAD) (λ: 190–600 nm), autosampler (injection volume 10 μL) and column oven (40 °C) from Dionex Softron GmbH, Part of Thermo Fisher Scientific at Dornierstrasse 4, D-82110 Germering, Germany. The separations were performed using a Synergi MAX-RP 100A column (50 × 2 mm, 2.5μ particle size) with a H_2O (+0.1% HCOOH) (A)/acetonitrile (+0.1% HCOOH) (B) gradient (flow rate 500 μl/min, injection volume 5 μl). Samples were analyzed using a gradient program as follows: 95% A isocratic for 1.5 min, linear gradient to 100% B over 6 min, after 100% B isocratic for 2 min, the system returned to its initial condition (90% A) within 1 min, and was equilibrated for 1 min.

2.4.3. Identification of peaks

The identification of constituents was performed by UPLC–DAD–MS analysis and by comparing the UV, MS spectra and fragmentation of the peaks in the samples with those of compounds from plants reported in the literature using the database (Scifinder, NIST/EPA/NIH Mass Spectral Library (NIST 14) and MassBank of North America (MoNA).

2.5. Antiparasitic test

In vitro tests on *H. contortus* were performed as described by Rabel et al. [**14**] on larval migration and Hounzangbé-Adoté et al. [**4**] on adult worm motility. The plant extracts and fractions were tested at different concentrations (depending on the test) ranging from 2400 μg/mL to 75 μg/mL with half dilution.

2.6. Antioxidant activity

2.6.1. 2,2′-diphényl-1-picrylhydrazyl (DPPH) method

The test was performed according to the method described by Lamien-Meda et al. [13]. A volume Vi (0.5 mL) of the extract was mixed with a volume Vs (2 mL) of ethanolic solution of DPPH of concentration 0.04 μg/mL. After the homogenization, the mixture was incubated for 30 min in the dark and the absorbance was read at 517 nm using a spectrophotometer against a blank (control containing all reagents except the test extract). Each test was repeated twice.

The IC_{50} value is determined by extrapolation from the regression line of absorbances versus concentrations (C).

The EC_{50} is defined as the effective concentration of the substrate that causes the loss of 50% of the DPPH activity and takes into consideration the concentration of DPPH in the reaction medium. It is the expression of the IC_{50} inhibitory concentration per milligram of DPPH. AntiRadical Potency (APR) is inversely proportional to the EC_{50} ($APR = 1/EC_{50}$).

A concentration range following a geometric dilution in 10 test tubes was prepared with ascorbic acid from 200 μg/mL to 0.39 μg/mL with vitamin C and then the test extract from 1 mg/mL to 0.0019 mg/mL. DPPH was prepared at 0.04 μg/mL.

2.6.2. Ferric reducing antioxidant power (FRAP) method

It was carried out according to the method of Hinneburg et al. [14] used by Bakasso, [15]. From the test solution (extract or ascorbic acid), 1 mL was taken to which 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 mL of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$] were added. After incubating the mixture for 30 min at 50 °C in a water bath, 2.5 mL of trichloroacetic acid (10%) was added and the whole mixture was centrifuged for 10 min at 3000 rpm. To 2.5 mL of the supernatant was added 2.5 mL of distilled water and then 0.5 mL of an aqueous $FeCl_3$ solution (0.1%).

Absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0–200 mg/l). The reducing power was expressed in ascorbic acid equivalents (EAA) (mmol ascorbic acid/g dry extract) considering 1 mM equals FRAP of 1 mL of the dry extract according to the following formula:

$$C = \frac{S \times D}{M \times Ci}$$

C = concentration of reducing compounds in mol EAA/g dry extract;
S = sample concentration read; D = dilution factor of the stock solution;
Ci = concentration of the stock solution; M = molar mass of ascorbic acid (176.1 g/mol).

2.7. Larval toxicity test

It allows the number of larvae surviving in the test solution prepared with seawater to be counted. The interest of this study is the existing correlation between shrimp larvae and human cells (lung cells, colon cells). This is a preliminary non-clinical toxicity test that was proposed by Michael et al. [16] and later developed by Vanhaecke et al. [17] and Solis et al. [18]**.** The protocol and design of the study on brine shrimp larvae (*Artemia salina*) was evaluated and validated firstly at the Laboratory of Pharmacognosy and Essential Oils and then by the thesis committee of the Doctoral School of Exact and Applied Sciences of the University of Abomey-Calavi.

2.8. Statistical analysis

The results were presented as mean ± standard deviation. The Excel spreadsheet was used to calculate the means, standard deviations and to generate some illustrative graphs. The different values were included in a two-way repeated measures analysis of variance models. The comparison of means for the different tests was done using the SNK procedure which runs the Student Newman and Keuls test in R software. The calculation of the IC_{50} and the comparison of the effect of the extracts is done with Graphpad software. The differences are considered significant at the 5% level.

3. Results and discussion

3.1. Results

3.1.1. Phytochemical screening

The phytochemical screening of the leaf extract of *M. inermis* collected from Zogbodomey reveals the presence of some major compounds such as alkaloids, catechic and gallic tannins, flavonoids, anthocyanins, leuco-anthocyanins, quinone derivative, steroids, triterpenoids, mucilage, reducing compound and the absence of saponosides, cyanogenic derivative and coumarin.

3.1.2. Qualitative determination of compounds contents of hydroethanolic extract from M. inermis using UPLC-QToF-ESI-MS

The identification of the constituents of the hydroethanolic extract prepared from the leaf powder of *M. inermis* was carried out by a rapid untargeted UHPLC-QTOF-ESIMS/MS-Based metabolomic profiling method described by Wouamba et al. [19] and the main results obtained are recorded in Table 1. Fifteen compounds were identified (Fig. 1 and Table 1) and then the structure of seven compounds could be determined, which are mitralactonal, rotundifoleine, isorotundifoleine, mitragynine, mitraciliatin, speciogynine, speciociliatin (or their isomers) (Fig. 2). These compounds were identified by comparing the UV, MS spectra, and fragmentations of the compounds with those reported in the literature based on compounds isolated from *Mitragyna* genus [[20,21]] and the database (Scifinder, NIST/EPA/NIH Mass Spectral Library (NIST 14) and MassBank of North America (MoNA).

3.1.3. Antioxidant activity of extracts

The free radical scavenging capacity assessed by the DPPH method allowed the determination of the inhibitory concentration IC_{50} which is an estimate of the antioxidant activity. It represents the dose of the sample that neutralizes 50% of the DPPH free radicals. As for the effective concentration (EC_{50}), the lower it is, the higher the antioxidant activity (Table 2). The results obtained show that the aqueous extract (EC_{50} = 69.66 μg/mg) has a better antiradical activity than the hydroethanolic extract (EC_{50} = 193.66 μg/mL). The FRAP method was used to measure the ability of the extracts to reduce Fe(III) ions to Fe(II). The results of this analysis showed that the aqueous extract (0.82 mmol g^{-1} EAA) has a more reducing capacity than the hydroethanolic extract (0.89 mmol g^{-1} EAA).

Table 1
Chromatographic analysis of the UPLC-QToF-ESI-MS spectrum of the hydroethanolic extract of *M. inermis*.

N°	RT (min)	[M + H]+ *m/z*	Molecular Formula (Cald. Mass)	name of the likely structure	Main fragments *m/z*	References
1	0.4	215.1222	$C_{11}H_{19}O_4$ (215.1283)	Not identified	215.12; 163.09; 140.10; 118.32; 110.04; 104.13; 84.98; 76.09	
2	1.5	365.1502	$C_{21}H_{21}N_2O_4$ (365.1501)	Mitralactonal	365.15; 351.28; 336.21; 335.17; 334.15; 303.13; 243.15; 219.16; 211.15; 191.19; 173.12; 158.04; 126.02	[22]
3	1.7	401.3129	$C_{22}H_{43}NO_5$	Not identified	401.31; 385.27; 383.24; 369.27; 341.25; 303.12; 285.20;	
4	1.8	401.3084	(401.3141)	Not identified	239.12; 224.18; 210.15; 208.04; 197.73; 158.04	
5	1.9	401.3126		Not identified		
6	2.0	401.3129		Not identified		
7	2.1	385.3127	$C_{26}H_{41}O_2$ (385.3107)	Not identified	369.27; 349.25; 325.19; 273.23; 256.19; 207.1; 163.1; 145.09; 162.02; 81.54	
8	2.2	417.3323	$C_{22}H_{45}N_2O_5$	Rotundifoleine[a]	399.29; 381.27; 371.29; 369.28; 367.26; 283.22; 237.16;	[23]
9	2.4	417.3323	(417.3328)	Isorotundifoleine[a]	236.48; 223.19; 193.14; 178.21; 163.10; 162.11	[23]
10	2.9	399.2289	$C_{23}H_{31}N_2O_4$	Mitragynine[b]	399.22; 397.31; 385.31; 383.29; 367.29; 365.28; 283.74;	[24]
11	3.0	399.2283	(399.2284)	Mitraciliatin[b]	281.23; 255.22; 239.20; 228.24; 223.19; 200.16; 199.19	[24]
12	3.5	399.2214		Speciogynine[b]		[25]
13	3.8	399.2286		Speciociliatin[b]		[26]
14	5.0	593.4293	$C_{37}H_{57}N_2O_4$ (593.4318)	Not identified	553.50; 509.47; 481.44; 399.46; 367.37; 333.32; 223.19; 185.17; 145.02	
15	5.6	607.4485	$C_{38}H_{59}N_2O_4$ (607.4475)	Not identified	573.57; 496.46; 445.48; 399.46; 385.48; 341.44; 293.32; 271.33; 217.16; 145.02; 80.07	

[a] Compounds **8** and **9** maybe interchanged.
[b] Compounds **10**, **11**, **12** and **13** may be interchanged.

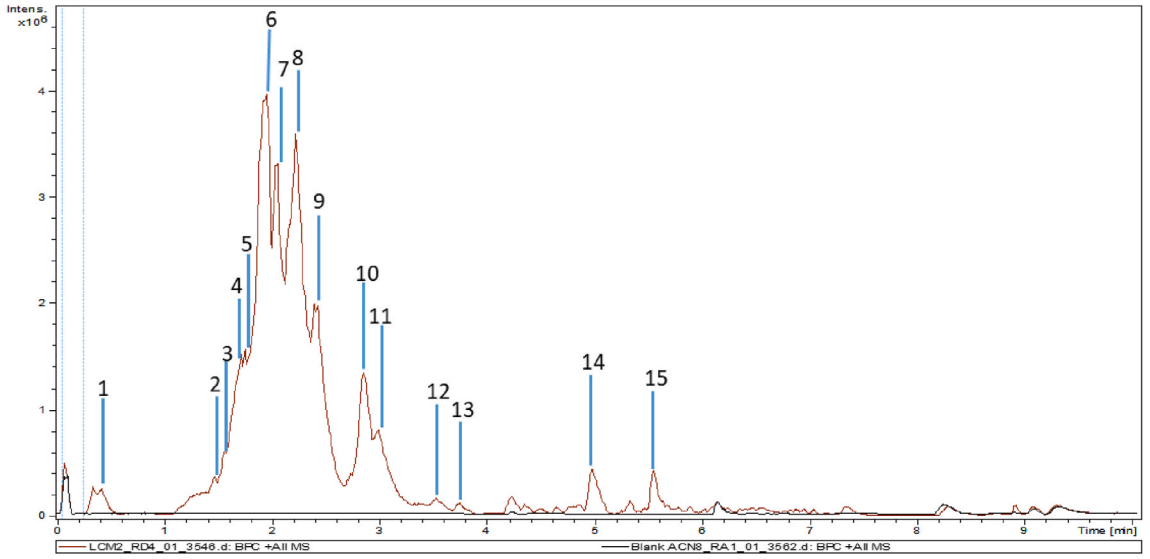

Fig. 1. UPLC-ESI-MS/MS chromatogram of *M. inermis* leaves total hydroethanolic extract (brown) and blank MeOH (black) in positive ion mode. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

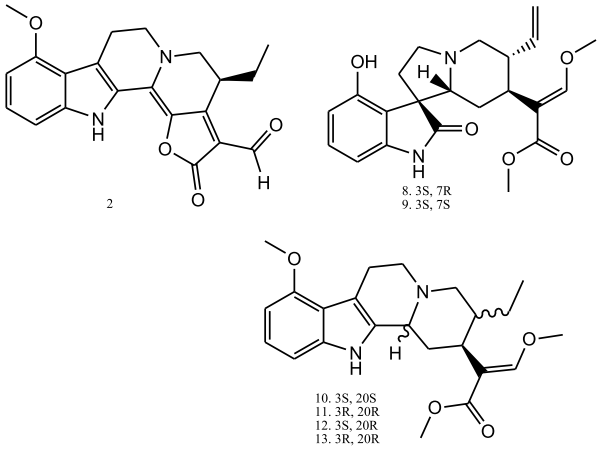

Fig. 2. Structures of the identified compounds.

Table 2
Antioxidant activity of extracts.

	Type of extract	DPPH method			FRAP method (mmol ascorbic acid/g dry extract)
		IC_{50} (μg/mL)	EC_{50} (μg/mg)	APR	
M. inermis	Aqueous	2.09	69.66	0.014355	0.82
	Hydroethanolic	5.81	193.66	0.005163	0.89
Vitamin C		0.48	1824.83	0.000547	0.0057

3.2. Larval toxicity test

The lethal concentration (LC_{50}) for which half of the larvae could not survive was determined for each extract using Fig. 3. The LC_{50} of the aqueous extract is 1.11 mg/mL and that of the hydroethanolic extract is 1.10 mg/mL.

3.2.1. Larval migration inhibition test

The aqueous and hydroethanolic extracts of *M. inermis* significantly inhibited *in vitro,* together with levamisole as a positive reference control, the migration of *H. contortus* larvae ($P < 0.001$) (Fig. 4). The inhibitory effect of the extracts was not dose dependent

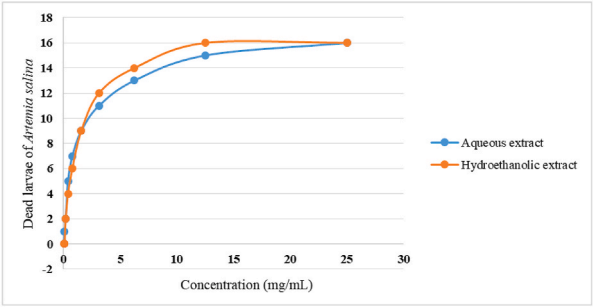

Fig. 3. Number of dead larvae as a function of extract concentration.

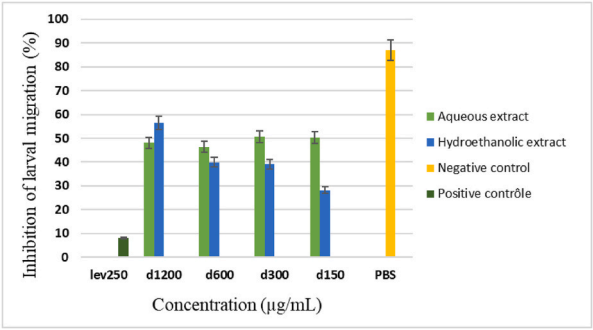

Fig. 4. Inhibition of larval migration of *M. inermis* extracts as a function of concentration.

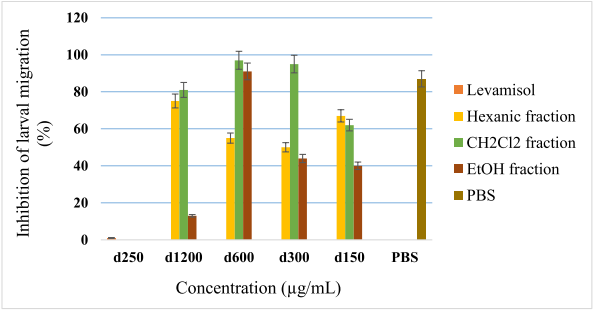

Fig. 5. Inhibition of larval migration of hexane, CH_2Cl_2 and EtOH fractions.

($P > 0.05$) nor was it solvent dependent ($P > 0.05$). However, the hydroethanolic extract of *M. inermis* appeared to be more active than the aqueous extract.

The larval migration rate of the negative control (PBS) was approximately 87% compared to the positive control (levamisole) which was very effective and almost inhibited all *H. contortus* larvae (1%). Statistical analysis indicated that no fraction was able to inhibit larval migration as did the positive control. Also, the inhibitory effect of the fractions did not differ from PBS ($P > 0.05$) (Fig. 5). Although there was no variation related to the fractions and their dose ($p > 0.05$), the ethanolic fraction seemed to show the best inhibition.

3.2.2. Inhibition of adult worm motility

For a period of 30 h, the adult worms were exposed to the different solutions of the test extracts at different concentrations (Table 3). The motility of *H. contortus* adult worms was significantly inhibited *in vitro* by levamisol (10.41%), aqueous extract (25%) and hydroethanolic extract (27.08%) of *M. inermis* compared to the PBS negative control (62.5%). The inhibitory effect varied with time ($p < 0.001$), and treatment doses ($p < 0.001$). Furthermore, the best inhibition results for the extracts were obtained at high doses, 2400 µg/mL and 1200 µg/mL respectively for the hydroethanolic extract and the aqueous extract.

Rapid effects on worm motility were observed with the hexane fraction at doses of 150 and 1200 µg/mL, and the dichloromethane fraction at a dose of 75 µg/mL after 6 h of contact, where almost total inhibition of adult worms was observed. It was not until 18 h that total inhibition was observed with the ethanol fraction at doses of 1200, 600 and 75 µg/mL (Table 4).

Table 3
Effects of aqueous and hydroethanolic extracts of *M. inermis* on adult *H. contortus* worms.

Motility of adult worms						
		Incubation time				
	Dose	6 h	12 h	18 h	24 h	30 h
Negative and positive control						
PBS	–	100	100	66,66	33,33	0
Lev	d500	50	0	0	0	0
	d250	66,66	0	0	0	0
	d125	0	0	0	0	0
Extracts from *M. inermis* sheets						
Aqueous	d2400	100	25	12,5	0	0
	d1200	100	25	0	0	0
	d600	100	62,5	0	0	0
	d300	100	50	12,5	0	0
	d150	100	62,5	0	0	0
	d75	100	50	0	0	0
Hydroethanolic	d2400	100	0	0	0	0
	d1200	100	75	0	0	0
	d600	100	50	0	0	0
	d300	100	75	0	0	0
	d150	100	75	0	0	0
	d75	100	75	25	0	0

Table 4
Effects of hydroethanolic extract fractions of *M. inermis* on adult *H. contortus* worms.

Motility of adult worms						
		Incubation time				
	Dose	6 h	12 h	18 h	24 h	30 h
Negative and positive control						
PBS		100	66,66	33,33	16,66	0
Lev	500	50	50	0	0	0
	250	66,66	0	0	0	0
	125	0	0	0	0	0
Hexane	2400	33,33	0	0	0	0
	1200	0	0	0	0	0
	600	33,33	0	0	0	0
	300	33,33	0	0	0	0
	150	0	0	0	0	0
	75	100	0	0	0	0
CH_2Cl_2	2400	66,66	0	0	0	0
	1200	100	0	0	0	0
	600	66,66	0	0	0	0
	300	75	25	0	0	0
	150	100	0	0	0	0
	75	0	0	0	0	0
EtOH	2400	100	50	0	0	0
	1200	100	100	33,33	33,33	0
	600	100	50	0	0	0
	300	100	66,66	66,66	33,33	0
	150	100	66,66	33,33	0	0
	75	100	100	0	0	0

In general, the fractions obtained from the hydroethanolic extract of *M. inemis* inhibited the motility of adult *H. contortus* worms ($p < 0.001$). The inhibitory effect was more remarkable with the hexane fraction which statistically showed the same inhibitory power as levamisol at the dose of 125 μg/mL.

3.3. Discussion

M. inermis is a plant widely used in traditional medicine for its many properties [[21,27]]. For its valorization, within the framework of this work, the anthelmintic and antioxidant potentials were evaluated. The leaf powder of the plant collected in Zogbodomey (in the Lama forest in Benin), was used for phytochemical screening and for the preparation of aqueous and hydroethanolic extracts. Evaluated for anthelmintic activity, the aqueous and hydroethanolic extracts of *M. inermis* inhibited *in vitro* migration of infesting larvae ($P < 0.001$) and motility of adult worms of ($P < 0.001$) *H. contortus* compared to the negative reference control (PBS).

Larval migration inhibition assays were performed to assess the penetration of L3 larvae into the gastric mucosa after contact with the extract [28]. This is reflected *in vitro* by the passage of L3 through very fine and smaller diameter sieve meshes after contact with the tested extract. The inhibitory effect of the extracts on larval migration was non-dose-dependent ($P > 0.05$) and did not vary with the extraction solvent ($P > 0.05$) although the hydroethanolic extract is more effective than the aqueous extract. These results corroborate those obtained with methanolic and acetone extracts which reduced the migration of *H. contortus* larvae with an inhibition ranging from 21.22 to 61.74% [**9**]. As well, these results are similar to those obtained by Tchetan et al. [10], who found that acetone extract affected the exsheathment kinetics of *H. contortus* L3s larvae ($p < 0.001$). These results are in favour of the aqueous and hydroethanolic extracts used in this work which, being prepared with solvents available to the population, could help breeders in the application of our results on *H. contortus* larvae.

As for the adult worms, they represent the pathogenic stage of the nematodes. The disruption of their motility when exposed *in vitro* to the extracts would result in feeding, mating and egg laying difficulties, thus ending the life cycle of these parasites and their elimination [4]. The inhibitory effect of the extracts on the motility of adult worms only varied over time ($P < 0.001$). These results are superior to those obtained by Alowanou et al. [9] with methanolic and acetone extracts of *M. inermis* on the motility of *H. contortus* adult worms. These authors found that at 24 h it was only at the dose of 2400 μg/mL that the adult worms could be totally inhibited whereas at 12 h we obtained this result with the hydroethanolic extract and at 24 h almost all larvae were inhibited with both extracts (Table 3).

As the EtOH/H_2O extract was more active on larvae and adult worms of *H. controtus* than the aqueous extract, it was used for solid-liquid partitioning to obtain the hexane, dichoromethane and ethanol fraction respectively. These three fractions were tested on the two pathogenic stages of the parasite. Statistical analysis showed that none of the fractions was able to significantly inhibit larval migration compared to PBS ($p > 0.05$). However, the overall inhibition percentages in decreasing order were 45% (ethanolic fraction), 61.75% (hexane fraction), 84.25% (dichloromethane fraction) and 87% (PBS).

On adult worms, the hexane fraction significantly inhibited, as did the positive control, the motility of adult worms ($p < 0.001$). The effect of the inhibition was very remarkable after 12 h of contact of the fraction with the parasite. Paradoxically, the ethanolic fraction, which inhibited more larval migration, was almost ineffective on adult worm motility. The anthelmintic activity of the plant could therefore be linked to the synergistic effect of the compounds present in the plant.

The antioxidant activity was evaluated *in vitro* by two different methods. By the DPPH method, the aqueous extract ($IC_{50} = 0.82$ μg/mL) of *M. inermis* leaves was found to be more active in scavenging DPPH free radicals than the hydroethanolic extract ($IC_{50} = 0.89$ μg/mL). This result was approved by the FRAP method where the extract reduced more Fe^{3+} ion to Fe^{2+} ion. From these results, we can say that the aqueous extract seems to present a strong antioxidant activity than the hydroethanolic extract. This could be explained by the polarity of water more than the EtOH/H_2O mixture confirming the influence of the solvent on the extraction of bactifs compounds [**9**]. If the antioxidant activity is linked to the presence of polyphenols, flavonoids and tannins [29] as well as the anthelmintic activity [30], we can pretend at first that the anthelmintic effect of the aqueous extract is linked to phenolic compounds. But, alkaloids will not remain insensitive in the activity that the extracts present, especially since *M. inermis* is a species of the Rubiaceae, a family known for its richness in alkaloids. This is revealed by the few compounds identified in the hydroethanolic extract by LC-MS.

The interpretation of the results of the analysis of the hydroethanolic extract by UPLC-ESI-MS in positive ionization mode allowed the identification of seven compounds based on the compounds isolated from the *Mitragyna* genus.

Little information exists in the literature on the antiparasitic activities of these seven alkaloids identified in the hydroethanolic extract. It would be interesting to isolate them in the future to explore their anthelmintic properties. However, some identified alkaloids *viz* mitragynine and speciofoliation have antinociceptive and analgesic properties which could explain the antioxidant activity observed [[26,31]].

Another way to enhance the value of this plant is to study its toxicity. Thus, the toxicity of aqueous and hydroethanolic extracts of *M. inermis* was evaluated *in vitro* on brine shrimp larvae (*Artemia salina*). The lethal concentration of the extracts was found to be above 0.1 μg/mL. According to the correspondence scale established by Mousseux [32], none of the extracts is toxic on these brine shrimp larvae and by ricochet on the 9 PS and 9 KB (human nasopharyngeal carcinoma) cells on the one hand and on the other hand the A-549 cells of the lung carcinoma and finally the HT629 cells of the colon carcinoma following the correlation which was established between the shrimp larvae and these different cells [33,34].

These different results may justify, in part, the use of this plant in traditional veterinary medicine for the sanitary management of parasitic small ruminants.

4. Conclusion

The aim of the present study is to use the bio-guided approach and UPLC-QToF-ESI-MS analysis to identify substances that might be responsible for *M. inermis* anthelmintic activity. The aqueous and hydroethanolic extracts of *M. inermis* leaves showed potent inhibitory activity compared to the hexane, dichloromethane and ethanolic fractions. This activity might be attributed to the synergistic effect of the compounds present in these extracts. The antioxidant properties of the extracts would help in decreasing the oxidative stresses that could weaken the animal and create a favorable environment for the proliferation of the parasite. The *in vitro* anthelmintic activity and the absence of a toxic effect on *A. salina* of *M. inermis* extracts could justify the traditional use of the leaves of this plant by breeders in traditional veterinary medicine.

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Author contribution statement

Placide Mahougnan Toklo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Georcelin G. Alowanou, Steven Collins N. Wouamba, Fidèle M. Assogba, Mathias A. Ahomadegbe, Simeon Fogue Kouam: Analyzed and interpreted the data; Wrote the paper.

Amoussatou Sakirigui: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bruno Ndjakou Lenta: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sylvie Hounzangbe-Adote, Joachim Djimon Gbenou: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Eléonore C. Yayi-Ladekan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors have no interests to declare.

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