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Anthelmintic and Antimicrobial Activities of Tannin Extracts of *Mitragyna inermis* (Willd.) Kuntze (Rubiaceae) and *Combretum glutinosum* Perr. ex DC (Combretaceae)

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Abstract: *Combretum glutinosum* and *Mitragyna inermis* are two plants used in Benin as a dewormer and antibiotic in traditional human and veterinary medicine. This study aimed to evaluate the anthelmintic, antimicrobial and antioxidant activities of the both plants tannin extracts. The extracts were tested *in vitro* on *Haemonchus contortus* larvae and worms and on the growth of 11 reference strains by agar medium diffusion method. Their chemical compositions were determined by UPLC-DAD-ESI-MS. It was found that total tannins extracted from plant leaves showed a strong inhibition on *H. contortus* larvae and adult worms compared to the negative control. Concerning the antimicrobial activity, *M. inermis* extract had an effect only on *Pseudomonas aeruginosa* with a MIC of 2.5 mg/mL. *C. glutinosum* extract inhibited the growth of most microbial strains with MIC values ranging from 1.25 to 20 mg/mL. The DPPH test showed that the extracts of *C. glutinosum* (IC_{50} = 8.04 µg/mL) and *M. inermis* (IC_{50} = 11.21 µg/mL) have good antioxidant activity and these results are confirmed by the FRAP method. Four (4) compounds could be identified in the tannin extract of *C. glutinosum* and could explain its activities. The results obtained from this work revealed that the tannins extracted from *C. glutinosum* showed better anthelmintic, antimicrobial and antioxidant activities compared to the extracts from *M. inermis*.

Keywords: *M. inermis*, *C. glutinosum,* Total Tannins, Anthelmintics, Antimicrobials, Antioxidants, LC-MS

1. Introduction

From the very beginning, humans have used plants for purposes other than food [1]. Whether the plant is edible or poisonous, whether it is used to resist the enemy or to heal, man has discovered through a series of failures and successes, the use of plants for his well-being [1]. Used since the dawn of time, medicinal plants constitute an inexhaustible source of substances with a wide variety of biological and pharmacological activities [2]. Anthoula, (2003) [1] estimate

that herbal medicines have been widely used in health care around the world since the earliest days of the human species and are of considerable importance in international trade. In Africa, up to 80% of the population relies on traditional medicine to solve their health problems [3]. Hence the richness of the African pharmacopoeia which is due to the diversity of human groups, languages, customs, and especially the ecological characteristics of the regions [4]. In Benin, there are no hamlets, no small villages without traditional healers or traditional therapists [5]. Thus, man discovered several beneficial experiences with plants before the appearance of synthetic drugs, which, after having won the market for several years, ended up showing limits. These include the high cost of these products for the rural poor, their inaccessibility and, above all, drug resistance to pathogens. The search for other control alternatives knew it was essential. In order to face the public health problems of our day's recourse to readily available local resources would be a real solution due to the fact that humanity is confronted with diseases of all kinds (microbial, parasitic, viral diseases, etc.) and dealing with health issues has proven to be a real societal problem especially in developing countries [6]. Thus, *M. inermis* and *C. glutinosum* are two plants of the Beninese flora known for their uses in human medicine in the treatment of some microbial diseases [7-9] and in veterinary medicine in the treatment of gastrointestinal strongles of small ruminants [10, 11]. The present study aims to evaluate the effect of tannic leaves extracts of both plants on *H. contortus* and on different multi-resistant microbial strains.

2. Materials and Methods

2.1. Microorganisms Used

Three types of microorganisms were used for this work: They were chosen for their pathogenicity and their frequency of infection and/or infestation of humans and/or animals.

Parasite: Larvae and adult worms of *Haemonchus contortus;*

Yeast: Candida albicans MHMR

Gram positive (+) bacteria: Staphylococcus aureus ATCC 29213, *Staphylococcus epidermidis* T22695, *Micrococcus luteus* ATCC 10240, *Streptococcus oralis, Enterococcus faecalis* ATCC 29212;

Gram negative (-) bacteria: Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilus* A24974, *Proteus vulgaris* A25015, *Salmonella typhi* R 30951401.

2.2. Extraction and Identification of Chemical Composition

2.2.1. Extraction

Both plants were collected in Benin and then identified at the National Herbarium of the University of Abomey-Calavi where specimens were kept under the numbers AA 6713 / HNB and HY 241 / HNB respectively of *M. inermis* and *C. glutinosum*. After drying and spraying the leaves, the powders were used for extractions. Total tannins were extracted following the method used by Feknous *et al.* [12] reviewed in this work. Briefly, 50 g of vegetable powder was defatted by maceration in 1000 mL of hexane for 8 hours at 4°C in the dark for the removal of waxy and lipidic substances. After filtration, chlorophyll and lipids were removed in the filtrate and the recovered pomace was again macerated for 8 hours at 4°C in the dark in 1000 mL of ethyl ether for the removal of the waxy and lipidic substances. The mixture was then filtered to remove phenols, catechins and oxybutyric acid. Finally, the pomace was taken up in 1 little of a hydro-alcoholic solution (75% ethanol-water) for maceration for 24 hours at 4°C in the dark to dissolve the tannins. The filtrate was recovered and then evaporated under vacuum at a temperature between 45 and 50°C. The residue obtained represents the raw tannin extract used for the tests.

2.2.2. Phytochemical Screening

The qualitative study of the hydrolysable and condensed tannins and then of some secondary metabolites, was carried out on the extracts following the method of Houghton and Raman [13].

2.2.3. Comparative TLC of Extracts with Standards

The chromatographic profile of the tannin extracts of *M. inermis* and *C. glutinosum* were analysed by TLC together with six (6) reference standards (caffeic acid, rutin, pyrogallol, ellargic acid, gallic acid, catechin) obtained from Sigma Aldrich through the Catholic University of Leuven. The stationary phase consisted of a plastic plate covered with silica gel 60 F254 and the mobile phase of the $CHCl_3$/MeOH mixture at different polarities according to each compound and a few drops of acetic acid. The plates were visualised under a UV lamp at two wavelengths: 365 nm and 254 nm. The spraying was carried out separately with sulfuric vanillin, ferric chloride and 10% sulfuric acid. Each TLC was performed 3 times for one spray per reagent.

2.2.4. UPLC-DAD-ESI-MS Tandem Mass Spectrometry Analysis

High resolution mass spectra were obtained with a QTOF spectrometer (Bruker, Germany) equipped with a HESI source. The spectrum was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high accuracy mass measurements within 0.40 ppm using Na Formate as a standard. 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 UHPLC system (Thermo Fisher, USA) consisting of an LC pump, a diode array detector (DAD) (λ: 190-600 nm), an autosampler (10 l injection volume) and a column oven (40 °C). 5.00 µL of the extracts to be tested, previously prepared in analytical methanol at a concentration of 5 mg/mL and then filtered through a syringe-filter-membrane, were injected into the Dionex Ultimate 3000 HPLC UPLC-DAD/MS (Germany), used to per-form the analyses. Separations were performed using a Synergi MAX-RP 100A (50 X 2 mm, particle size

2.5 µ) 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 analysed using a gradient programme 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 state (90% A) in 1 min, and was equilibrated for 1 min. Data were analysed with Compass DataAnalysis software and sample identification was performed by comparing UV, MS and peak fragmentation spectra in the samples with those of compounds reported in the literature using the Scifinder database, the NIST/EPA/NIH mass spectral library (NIST 14) and MassBank of North America (MoNA).

2.3. Antiparasitic Test

2.3.1. Evaluation of Larval Migration Inhibition

It was performed at different concentrations of the tested extract on infesting *H. contortus* larvae following the method proposed by Rabel *et al*. [14] and used by Toklo *et al*. [15].

2.3.2. Motility Inhibition Technique for Adult Worms

It was carried out on adult worms of *H. contortus* following the method of Hounzangbe-Adote *et al*. [16] used by Toklo *et al*. [17]

2.4. Evaluation of Antimicrobial Activity

2.4.1. Antibiogram

The study of the sensitivity of the microorganisms to the extracts was carried out by the technique of diffusion in agar medium following the method described by Bauer *et al.* [18]. A bacterial pre-culture (1 colony in 1 mL of MH broth) from the previous day was diluted to a turbidity of 0.5 on the McFarland scale (i.e. 10^8 UFC/mL) and reduced to 10^6 UCF/mL. The petri dishes containing the solid medium (solid MH) were inoculated with 1 mL of the inoculum. Wells of about 6 mm diameter with 4 wells per box were made and impregnated with 30 µl of the extract solution (20 mg/mL). After 15 min prediffusion at room temperature, the Petri dishes were incubated at 37 °C for 24 hours. For each extract, the experiment was performed twice. Antimicrobial activity was determined using a ruler measuring the diameter of the microbial growth inhibition zone produced around the wells after incubation. A negative control (DMSO) at 0.4% and a positive control (gentamicin) were also tested.

2.4.2. Determination of the Minimum Inhibitory Concentration (CMI) and Bactericidal Concentration (CMB)

The liquid macro-dilution method reported by Delarras, [19] was used for the determination of the minimum inhibitory concentration. Then, referring to the results of the MIC test, all tubes showing no visible growth were identified and inoculated onto a Petri dish containing MH agar medium and incubated at 37°C for 24 hours. The lowest concentration of the extract where no growth was observed was considered the Minimum Bactericidal Concentration.

2.5. Antioxidant Activity

The DPPH test was carried out according to the method described by Lamien-Meda *et al*. [20]. For the FRAP method, the method of Hinneburg *et al*. [21] as described by Bakasso, [22] was used.

2.6. Statistical Analysis

The Excel spreadsheet was used to calculate the means, standard deviations and generate the illustrative graph. The comparison of the anthelmintic effect according to dose, time and plant was done using the SNK procedure using the agricolae package of the R software and then the Graphpad software (two-way ANOVA Multiple comparisons) for the calculation of the IC_{50} of larval migration. Differences are considered significant at the 5% threshold.

3. Results and Discussion

3.1. Phytochemical Profile

The qualitative chemical study of the extracts of both plants revealed the presence of hydrolysable tannins, condensed tannins, flavonoids and the absence of anthocyanins, leuco-anthocyanins and coumarins.

The presence of flavonoids in specific tannin extracts could be explained by the fact that the condensed tannin units are mainly linked by C4-C6 or C4-C8 bonds of flavan-3-ol and flavan-3,4-diol derivatives [23]. These bonds can easily break down in a chemical reaction to give flavonoids.

3.2. Compound Identification

Of the five standards (caffeic acid, rutin, pyrogallol, ellargic acid, gallic acid, catechin) used on TLC in comparison with the tannin extracts of the two plants, none of the standards presents the same frontal ratio as the spots obtained for the two extracts. We can deduce that the extracts certainly do not contain these used standards.

Analysed by UPLC-DAD-MS, the tannin extract of *M. inermis*, presented four peaks (figure 1) of which none were identified (table 1). This result can be justified by the fact that *M. inerms* is a plant of the Rubiaceae family known to be rich in alkaloids. The four peaks identified will probably be new polyphenols that it would be interesting to isolate later. As for the tannin extract of *C. glutinosum*, it presented eleven peaks (figure 2) of which four could be identified (table 2) as the 1-O-galloyl-6-O-(4-hydroxy-3,5-dimethoxy)benzoyl-beta-D-glucose [24], 2,3-(S)-hexahydroxydiphenoyl D-glucose [25], punicalin [25] and punicalagin [25] (figure 3). The compounds were identified on the basis of HPLC-DAD-HRESI-MS data, their UV spectra and by comparison with literature data using scifinder, massbank etc. The last three compounds show no inhibited hepatitis B virus surface antigen while 1-O-galloyl-6-O-(4-hydroxy-3,5-dimethoxy)benzoyl-beta-D-glucose exhibited potent hepatoprotective activity [24].

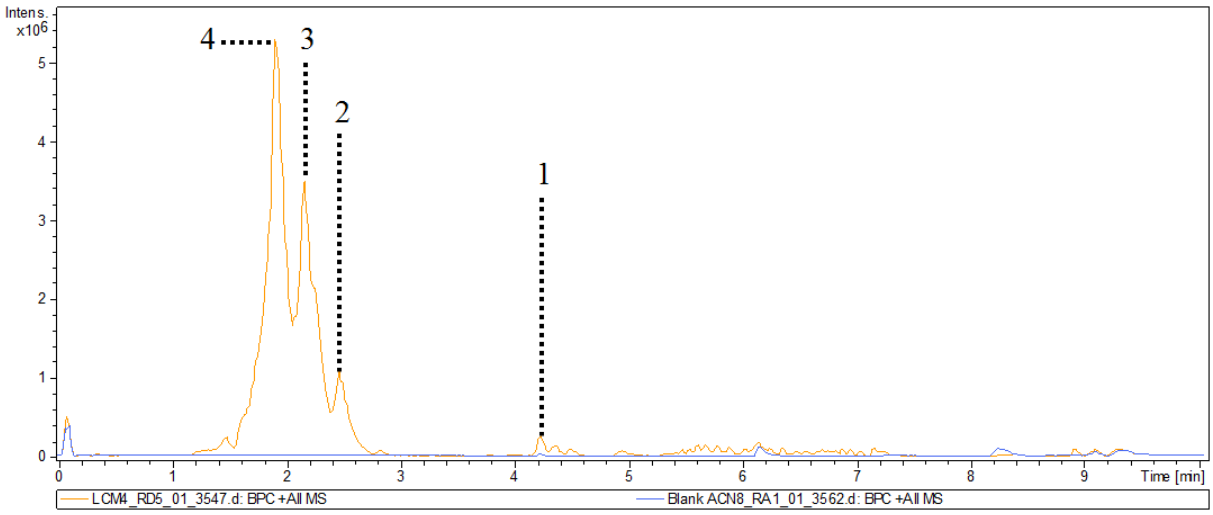


Figure 1. *Chromatographic profiles of M. inermis tannic extract.*

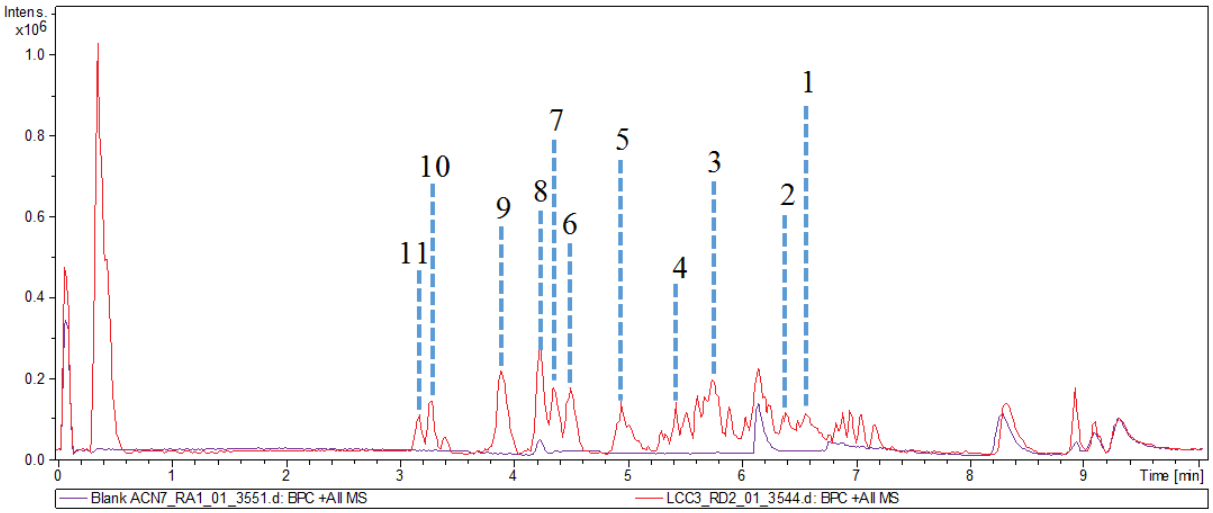


Figure 2. *Chromatographic profiles of C. glutinosum tannic extract.*

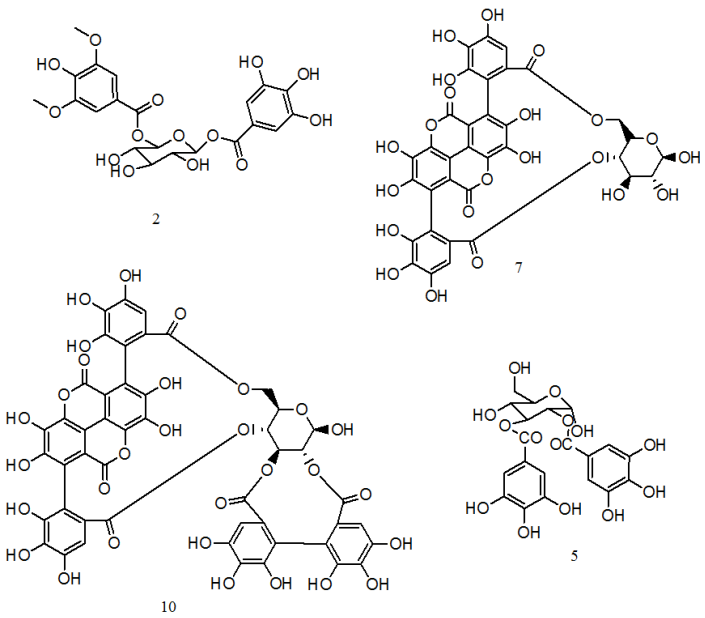


Figure 3. *Identified compounds of the tannin extract from C. glutinosum.*

Table 1. *FT-MS ions and molecular formular of compounds in M. inermis tannic extract.*

N°	RT [min]	$[M+H]^+$		Molecular Formular	Mains fragments	Name of Compound
		Exp.	Calc.			
1	4.2	579.4448	579.4472	$C_{31}H_{63}O_9$	579.44; 459.45; 371.37; 301.22; 205.14; 149.06	Not Identified
2	2.5	431.3342	431.3373	$C_{24}H_{47}O_6$	431.33; 401.31; 203.16; 181.17	Not Identified
3	2.2	801.6105	801.6092	$C_{45}H_{85}O_{11}$	801.61; 431.33; 417.31; 401. 31; 385.30; 343.29; 190.14	Not Identified
4	1.9	401.3156	401.3267	$C_{23}H_{45}O_5$	401.31; 399.29; 385.28; 369.28; 242.20; 197.13; 160.12	Not Identified

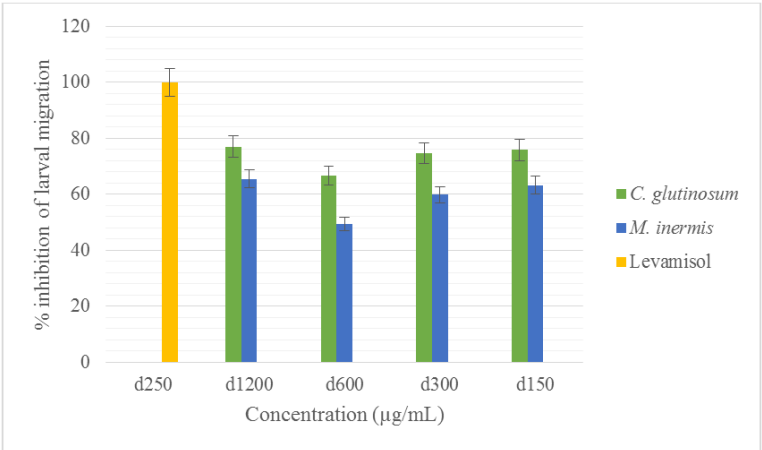
RT: retention time**,** *Exp:* Experimental mass, *Calc:* Calculated mass.

Table 2. *Main signals of compounds detected in C. glutinosum tannic extract.*

N°	RT [min]	$[M+H]^+$		Molecular formular's	Mains fragments	Compound name's	References
		Exp.	Calc.				
1	3.2	769.3937	769.401	$C_{39}H_{61}O_{15}$	769.39; 739.37; 686.52; 453.45; 381.19; 359.20; 239.19	Not Identified	
2	3.3	499.3957	499.1088	$C_{21}H_{23}O_{14}$	499.39; 481.38; 453.45; 411.21; 389.22; 359.20; 345.18; 315.27; 283.33; 243.62	1-O-galloyl-6-O-(4-hydroxy-3,5-dimethoxy)benzoyl-beta-D-glucose	[24]
3	3.9	911.2599	911.2575	$C_{26}H_{55}O_{34}$	911.25; 903.25; 845.23; 753.66; 627.50; 611.51; 529.48; 451.43; 343.77	Not Identified	
4	4.2	579.4377	577.4316	$C_{31}H_{61}O_9$	479.43; 527.46; 467.43; 383.30; 319.30; 301.22; 205.14; 149.06	Not Identified	
5	4.4	485.4670	485.0931	$C_{20}H_{21}O_{14}$	485.46; 467.43; 419.38; 375.34; 359.21; 331.30; 315.34; 301.21; 234.20; 207.23.	2,3-(S)-hexahydroxydiphenoyl D-glucose	[25]
6	4.5	443.4453	443.4464	$C_{28}H_{59}O_3$	443.44; 419.38; 375.34; 339.33; 305.32; 283.33; 265.32; 181.67	Not Identified	
7	5.0	783.7428	783.0681	$C_{34}H_{23}O_{22}$	783.74; 754.70; 738.14; 593.42; 549.50; 455.46; 399.46; 353.35; 299.27; 177.21	punicalin	[25]
8	5.5	1069.9718	1069.9586	$C_{65}H_{129}O_{10}$	1069.97; 943.99; 868.90; 749.71; 705.68; 661.64; 609.52; 483.50; 443.50; 383.41; 283.33; 143.14	Not Identified	
9	5.8	1013.9396	1012.9441	$C_{42}H_{13}O_{31}$	1013.93; 821.79; 621.46; 485.50; 397.43; 353.36; 284.37	Not Identified	
10	6.4	1087.0302	1087.09	$C_{48}H_{31}O_{30}$	1087.03; 1084.96; 1041.98; 997.95; 953.91; 859.82; 757.73; 717.72; 673.68; 598.55; 576.53; 427.49; 338.42; 275.23	punicalagin	[25]
11	6.6	1218.1491	1215.1374	$C_{54}H_{39}O_{33}$	1218.14; 917.87; 721.66; 642.59; 620.57; 585.60; 427.49; 101.10	Not Identified	

3.3. Anthelmintic Activity Inhibition of Larval Migration

Figure 4 shows the percentages of inhibition of larval migration. With levamisol, inhibition was almost total at a rate of 100%. For the tannic extract of *M. inermis*, the inhibition rate ranged from 49.42 to 65.51% while it varied from 66.66 to 77.01% for the tannic extract of *C. glutinosum*. Thus, like the positive reference control (levamisol), a significant inhibition effect was observed ($p < 0.001$) with both tannic extracts on the migration of *H. contortus* larvae. This inhibitory effect did not vary with plant or dose ($p > 0.05$).



d250 = Levamisole 250 µg/mL; d1200 = 1200 µg/mL; d600 = 600 µg/mL; d300 = 300 µg/mL; d150 = 150 µg/mL.

Figure 4. *Inhibitory effect of extracts on larvae.*

3.4. Anthelmintic Activity Inhibition of Adult Worms

Table 3. *Effect of tannic extracts on the motility of adult worms.*

	Dose	6h	12h	18h	24h	30h	36h
PBS	d0	100	100	87.5	62.5	50	0
Lev	d500	0	0	0	0	0	0
	d250	0	0	0	0	0	0
	d125	12.5	0	0	0	0	0
Tannic extract of *C. glutinosum*	d2400	75	50	12,5	12,5	0	0
	d1200	75	37,5	0	0	0	0
	d600	100	50	25	0	0	0
	d300	75	50	25	12,5	0	0
	d150	100	62,5	37,5	25	0	0
	d75	100	50	25	25	0	0
Tannic extract of *M. inermis*	d2400	100	50	12,5	12,5	0	0
	d1200	100	25	12,5	12,5	0	0
	d600	100	37,5	37,5	25	0	0
	d300	75	50	50	37,5	0	0
	d150	100	87,5	25	25	0	0
	d75	100	62,5	25	25	0	0

The rapid effect of inhibition of adult worm motility after 6 hours of contact was not observed with an extract like the positive references. However, extracts inhibited adult worm motility compared to the negative reference control ($p < 0.001$). The effect of inhibition was dose dependent ($p < 0.001$) and time-dependent ($p < 0.001$). Similarly, the inhibitory effect of *C. glutinosum* is significantly different from the inhibitory effect of *M. inermis* ($p < 0.05$) and *C. glutinosum* tannic extract appears to be more effective than *M. inermis* tannic extract.

Following the various public health problems encountered through the toxicity of certain chemicals and their side effects and the emergence of parasites resistant to many conventional drugs, many studies have focused on the exploitation of natural bioactive molecules as an alternative for the treatment of many human and animal diseases [26, 27]. The secondary metabolites, in this case the polyphenols present in medicinal plants, give them interesting properties in pharmacognosy and phytotherapy. These polyphenols are molecules originating from the plant kingdom and manufactured exclusively by plants [28]. They are commonly subdivided into tannins, lignins, flavonoids, anthocyanins…, all of which derive from the assembly of phenolic units, with several biological properties [29]. Tannins, on the other hand, are known for their anthelmintic and antimicrobial properties. Indeed, condensed tannins are particularly suspected to be responsible for anthelmintic properties [27, 30]. It appears that total tannin extracts of *C. glutinosum* and *M. inermis* significantly ($p < 0.001$) reduce in *vitro* larval migration and lamility of adult worms of H. *contortus* as well as the positive reference control (levamisol) compared to PBS. The inhibitory potency of *C. glutinosum* appears to be higher than that of *M. inermis*. These results corroborate those of Alowanou *et al.* [31] who showed that acetone and methanol extracts of *C. glutinosum* and *M. inermis* inhibited *in vitro*, egg hatch, larval migration and adult worm motility of *H. contortus*. The presence of tannins could therefore contribute to the anthelmintic activity of these plants.

3.5. Antimicrobial Activities

Table 4 presents the variation in parameters used to assess the antimicrobial activity of tannin extracts of *C. glutinosum* and *M. inermis* on the microbial strains tested.

Table 4. *Antimicrobial activity of crude tannic plant extracts.*

		S. aur	*P. aer*	*P. mir*	*M. lut*	*S. epi*	*P. vul*	*S. ora*	*E. fae*	*E. coli*	*C. alb*	*S. typ*
MI	DI (mm)	-	17,5 ± 3,53	-	-	-	-	-	-	-	-	-
	CMI (mg/mL)	nd	2,5	nd	nd	nd	nd	nd	nd	nd	nd	nd
	CMB (mg/mL)	nd	20	nd	nd	nd	nd	nd	nd	nd	nd	nd
	CMB/CMI	nd	8	nd	nd	nd	nd	nd	nd	nd	nd	nd
CG	DI (mm)	18,5 ± 4,94	10 ± 0,0	21 ± 1,41	11 ± 0,0	12,5 ± 0,7	-	20 ± 0,0	-	-	-	8 ± 11,3
	CMI (mg/mL)	5	1,25	5	20	10	nd	2,5	nd	nd	nd	20
	CMB (mg/mL)	20	5	5	20	20	nd	20	nd	nd	nd	> 20
	CMB/CMI	4	4	1	1	2		8	nd	nd	nd	nd
CM	DI (mm)	25	26	15	26	28	25	14	24	15	-	31

S.aur = *Staphylococcus aureus*; P. aer = *Pseudomonas aeruginosa*; P. mir = *Proteus mirabilus*; M. lut = *Micrococcus luteus*; S. epi = *Staphylococcus epidermidis*; P. vul = *Proteus vulgaris*; S. ora = *Streptococcus oralis*; E. fae = *Enterococcus faecalis*; E. coli = *Escherichia coli*; C. alb = *Candida albicans*; S. typ = *Salmonella typhi*; MI = *M. inermis*; CG = *C. glutinosum*; DI = Diameter of inhibition; CMI = Minimum Inhibitory Concentration; CMB = Minimum Bactericidal Concentration; nd = Not determined; - = Not active, CM = Gentamicin.

The antibiotic susceptibility test is a test that can be used to determine the sensitivity of strains to the extracts to be

tested. The inhibition zone measured in mm allows to assess the effectiveness of the antibiotic. The higher of inhibition diameter is the more effective extract. The tannic extracts of both plants were tested at a single concentration of 20 mg/mL. Only the extracts that showed activity at this concentration were used to find the Minimum Inhibitory Concentration (CMI) and Minimum Bactericidal Concentration (MBC).

Among the 11 strains tested, it appears that only *Pseudomonas aeruginosa* strain was sensitive to the tannic extract of *M. inermis*, the other strains developed resistance against the tannic extract of *M. inermis*. Our results are somewhat similar to previous work that reported the resistance of different strains to *M. inermis* extracts. Indeed, the antibacterial activity evaluated on *Bacillus subtilis* and *Pseudomonas syringae* with the leaves, trunk bark and roots of *M. inermis* showed that only the hexanic extract of *M. inermis* leaves inhibited the microbial growth of *P. syringae,* while acetone extract from leaves and roots and then hydromethanolic (1:1) extract from trunk bark and hexanic extract from M. *inermis* inhibited the microbial growth of *B. subtilis* [32]. On *S. typhi*, our results do not corroborate with those of [33] who obtained in Ghana an inhibition diameter ranging from 9 mm to 19 mm with hydroethanolic and aqueous extracts. This difference in result could be due to the area of harvest or especially to the type of extract, i.e. the variation in the chemical composition of the extracts. Our results are also in agreement with those of Wakirwa, [34] who had evaluated the effect of the methanolic extract on *S. aureus, E. coli and K. pneumoniae.* The CMI was 50 mg/mL on *S. aureus* and *E. coli* and 25 mg/mL on *K. pneumoliae*.

Antimicrobial tests with an extract of *M. inermis* were performed for the first time in this work on *P. aeruginosa, P. mirabilus, M. luteus, S. epidermidis, P. vulgaris, S. oralis, E. faecalis* and finally on *C. albicans*. Only in case of antibiotic resistance to *P. aeruginosa* would the tannic extract of *M. inermis* be a good candidate in the treatment of the infection.

Unlike *M. inermis* tannic extract, *C. glutinosum* tannic extract showed good antimicrobial activity on the majority of strains tested with a concentration of 20 mg/mL. With the CMB/CMI ≤ 4, the extract showed bactericidal power on *S. aureus*, *P. aeruginosa*, *P. mirabilus, M. luteus*, *S. epidermidis* and bacteriostatic power on *S. oralis* with the CMB/CMI > 4. In addition, the CMI is 20 mg/mL on *S. typhi* and finally, at 20 mg/mL, the tannic extract of *C. glutinosum* could not inhibit the growth of *P. vulgaris*, *E. faecalis, E. coli* and *C. albicans*. According to the work of Usman *et al.* [35], the saponic and flavonoid fractions of the methanolic extract of *C. glutinosum* bark at concentrations higher than those of our study (25, 50 and 100 mg/mL) showed antibacterial activity on *Bacillus subtilis, Corynae bacterium* spp., *E. coli, Shigella dysenteriae* and *Aspergillus niger.* Both fractions developed resistance against *Aspergillus niger* (CMI > 100 mg/mL) as well as the saponic fraction on *Bacillus subtilis.* Similarly, for the methanolic and aqueous extracts of trunk and leaf bark of *C. glutinosum* tested on *S. typhi*, *P. aeruginosa*, *S. aureus* and *E. coli*, only the methanolic extract of the leaves could not inhibit the growth of *P. aeruginosa* and *E. coli* [36]. The other extracts have antimicrobial activity with inhibition diameters ranging from 7 to 18 mm [36]. Thus, the tannic extract of *C. glutinosum* leaves would be a good antibiotic candidate in traditional medicine.

Table 5. *Antioxidant activity of crude tannic plant extracts.*

Plants	DPPH Method			FRAP method (EAA)
	IC_{50} (µg/mL)	EC_{50} (µg/mg)	APR	
C. glutinosum	8.04	268	0.003731	1.70
M. inermis	11.21	373.66	0.002676	1.32

Legend: EAA = mmol ascorbic acid /g dry extract.

3.6. Antioxidant Activity

In order to fully appreciate the antioxidant activity of extracts, two methods are used in this work for its evaluation. The first is the trapping of the free radical of diphenylpicrylhydrazyl of purple color in solution for the appearance of diphenylpicrylhydrazine of yellow color in solution: this is the method of DPPH (Yann-Olivier, 2015). And on the other hand, the ability of the extracts to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}): this is the FRAP method [21]. The results showed a powerful antiradical activity with IC_{50} of 8.04 and 11.21 and a reducing power of 1.7 and 1.32 respectively for the tannic extracts of *C. glutinosum* and *M. inermis*. In general, the tannic extract of *C. glutinosum* seems to have a higher antioxidant activity than that of *M. inermis*.

Indeed, the antioxidant activity of polyphenols, in this case tannins, is recognized and could explain their potential role in the prevention of several diseases [37]. The strong antioxidant power of *C. glutinosum* obtained could explain its stronger anthelmintic and antimicrobial activity. This plant is also recognized as a tannin plant [25] unlike *M. inermis,* which is an alkaloid plant of the Rubiaceae family.

The antioxidant activity of *C. glutinosum* extract could be related to the presence of 1-O-galloyl-6-O-(4-hydroxy-3,5-dimethoxy)benzoyl-beta-D-glucose, which has been identified in the extract and previously shown to have a potent hepatoprotective activity against $_{D}$-GalN/TNFa-α-induced cell death in primary mouse hepatocyte cultures. Further studies are needed to isolate this compound in order to assess its antioxidant capacity.

4. Conclusion

From the present study, we can retain that total tannins extracted from the leaves of *C. glutinosum* and *M. inermis*, showed *in vitro* an anthelmintic activity on two different stages of the parasite *H. contortus*. These extracts showed no

antifungal activity on *C. albicans*. However, the total tannins extracted from *C. glutinosum* leaves seem to inhibit the growth of several bacterial strains, unlike the tannin extract of *M. inermis* which only inhibited *P. aerosinga*. Both extracts also have strong antioxidant activity. Subject to in vivo testing and isolation of the identified compounds to evaluate their activities, these results confirm the use of these plants in human and veterinary medicine, and especially propose the leaves of *C. glutinosum* for the formulation of an antibacterial plant-based drug. Furthermore, the chromatograms obtained for both extracts indicate that these extracts can be a great source for the isolation of new compounds.

Conflicts of Interest

The authors declare they have no conflict of interest in this work.

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