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Antibacterial, antioxydant and antidiarrheal properties of *Tristemma hirtum* P. beauv.

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Abstract

Background: The aim of this study was to investigate the antibacterial, radical-scavenging and antidiarrheal properties of *Tristemma hirtum*.
Methods: The methanol extract of *Tristemma hirtum* was prepared by successive fractionation in hexane and ethyl acetate solvents. The phytochemical composition of crude extract and fractions was studied using standard methods. Their antimicrobial activities were evaluated against Gram-negative and Gram-positive species of pathogenic bacteria using the broth-microdilution method. The kinetics of 2,2 Diphenyl 1 picrylhydrazyl (DPPH$^{\bullet}$) free radical scavenging activity was also studied *in vitro*. The anti-diarrhoeal properties of the ethyl acetate fraction of *T. hirtum* were evaluated *in vivo* by the *Shigella*-induced and functional castor oil-induced diarrhoeal models in Wistar rats.
Results: The antibacterial assays showed that the crude extract and its fractions presented antibacterial activities with Minimum inhibitory concentrations (MICs) varying between 128 and 2048 µg/ml. The extracts also presented radical-scavenging activities with inhibitory percentages comparable ($p \leq 0.05$) to those of L-ascorbic acid against the DPPH $^{\bullet}$. The ethyl acetate fraction of *T. hirtum* presented the highest antibacterial activity on *S. flexneri* (with a MIC value of 128 µg/ml). The *in vivo* studies revealed that this fraction may inhibit shigella-induced diarrhoea in rats after six days of treatment. The same fraction almost completely reduced castor-oil diarrhoea for doses ≥ 400 mg/kg.
Conclusion: Methanol extract of *T. hirtum* and its fractions possess antimicrobial and antioxidant activities and confirm its usage in the treatment of various diseases such as infectious diseases and diarrhea.

Keywords: *Tristemma hirtum; antimicrobial; antioxidant; antidiarrheal; diarrhea; infectious diarrhea.*

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Background

Infectious diseases are one of the main causes of morbidity and mortality in the world [1] and primarily in developing countries where sanitary and financial conditions are very poor. These diseases are due to a large number of microorganisms and particularly bacteria which become more and more resistant to the available antibiotics [2]. Diarrheal diseases which could be infectious or functional kill 5-8 million peoples particularly children each year in the world [3]. Otherwise, Oxidative stress may cause many dangerous disorders such as mutagenesis, carcinogenesis, lipid peroxidation, oxidation and fragmentation of proteins [4, 5]. Most living species have an efficient defense mechanism to protect themselves against the oxidative stress induced by Reactive Oxygen Species [6]. But an imbalance of the body antioxidant defense system and free radical formation can appear after an over-production of free radicals [7]. Oxidative stress is responsible for a variety of degenerative processes in some human diseases such as gastrointestinal affections including diarrhea, an infection that has long been recognized as one of the most important health problems in developing countries [8].

The search for new therapeutic agents from natural sources has intensified in response to the limitations of currently available therapy and the emergence of drug-resistant strains. In our precarious socioeconomic conditions, it is important to screen the natural environment for the discovery of new bioactive compounds of high therapeutic efficacy and of low cost. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness [9]. They are produced in medicinal plants together with a wide range of bioactive molecules making them a rich source of different types of potential drugs [10-12]. Among medicinal plants, *Tristemma hirtum* P. Beauv is a forest shrub of 1.25 meter height, belonging to the family Melastomataceae which grows in marshy regions of African countries including Cameroon [13]. Antibacterial activities of *T. hirtum* was previously demonstrated against Gram-negative bacteria [14]. In Ivory Coast, it is used to regulate menstrual cycle and its fruits are used as food [15]. In the west-region of Cameroon, this plant is used in combination with other plants to treat typhoid fever, diarrheal diseases, hemorrhoids and food intoxications. This study was therefore, aimed at evaluating the antimicrobial, antioxidant and antidiarrheal activities of *T. hirtum* methanol extract and its fractions.

Methods

Plant material

Tristemma hirtum aerial parts were collected from the locality of Dschang, Cameroon in May 2009. The Identification was done at the National Herbarium (Yaoundé), using a voucher specimen registered under the reference No 12725/HNC.

Preparation of extracts

The plant was air-dried at room temperature under shade in the laboratory, chopped and pulverized using an electrical blinder to obtain fine powder. One kilogram of plant powder was macerated in 6L of methanol. The mixture was stirred daily and 48 hours later, the resulting solution were then filtered using Whatman paper N° 1 and the filtrate concentrated by evaporating the solvent at 65 °C using a rotatory evaporator (Buchi R-200) to obtain the extract. The process was repeated on the residues obtained above in order to maximize yield. The methanol extract was subjected to successive fractionation [16] in hexane and ethyl acetate solvents. Briefly, 100 g of methanol extract was successively partitioned in hexane and ethyl acetate for 2 hours at room temperature. These solvents were evaporated at 50°C to give the hexane (14.53g), ethyl acetate (5.87g), and residual (76.95g) fractions, respectively. The methanol extract and its fractions were stored in bottles at 4°C in the refrigerator till usage.

Microorganisms

Ten bacterial strains and isolates responsible for bacterial infections were collected from the Pasteur Centre in Yaoundé, Cameroon were used in this study. These included two strains of Gram positive bacteria *(Staphylococcus aureus ATCC25922 and Enterococcus faecalis ATCC10541),* four strains of Gram negative bacteria *(Escherichia coli ATCC11775*, *Klebsiella pneumonia ATCC13883*, *Pseudomonas aeruginosa ATCC27853*, *Salmonella typhi ATCC6539)* and four isolates of Gram negative bacteria (*Proteus mirabilis, Shigella flexneri, Salmonella paratyphi* A *and Salmonella paratyphi* B).

Experimental animals

Male rats of Wistar strain (8-10 weeks old; 150-175 g) were used for antidiarrhoeal activity. Prior to this study, rats were acclimatized for a period of one week. The animals were housed in plastic cages under normal laboratory conditions (12 hrs light/dark cycle: 22 ± 3°C), in the Experimental Animal House of the Department of Biochemistry, University of Dschang. Drinking water and feed were provided *ad libitum* to the animals [17].

Minimum Inhibitory Concentration (MIC) and Minimum bacteridal concentration (MBC) determination

The MICs of the crude extract and its fractions were determined by microdilution assay**[18]**: the test sample was first dissolved in DMSO. The solution obtained was completed by Mueller Hinton Broth (MHB) to obtain a final concentration of 16.384 g/ml followed by a two-fold serial dilution in a 96 wells microplate. 100 µl of the inoculum (2 x10^6 CFU/ml) prepared in MHB were then added. The plates were covered with a lead and incubated at 37°C for 18 h. Wells containing MHB, of the inoculum at 10^6 CFU/ml and 2.5 % DMSO (dimethyl sulfoxide) were used as negative control. The MICs were detected by the addition of 40 µl of INT (0.2 mg/ml) and incubation at 37° C for 30 minutes. The MIC was read as the lowest sample concentration that prevented this change and exhibited complete inhibition of microbial growth. Minimum bactericidal concentrations (MBCs) were determined by plating 10 µl of the content of each negative well on Mueller-Hinton Agar. MBCs were defined as the lowest concentration yielding negative subcultures. All the experiments were performed in triplicates. Ciprofloxacin at concentrations ranging between 0.25 and 32 µg/ml was used as positive control for antibacterial activities. The experiment was repeated twice in triplicates for both MIC and MBC determinations.

Radical-scavenging assay: DPPH assay method

The free radical scavenging activity of the crude extract and fractions were evaluated using a procedure previously described by Noumedem [19, 20] with slight modifications. Briefly, the test

samples were prepared in methanol and 100 µl of each sample added to 900 µl of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (a 20 mg/L DPPH) methanol solution, to give final concentrations of 50, 100, 200, 400 and 800 µg/ml. Ascorbic acid was used as a standard control. The content of each preparation was mixed and incubated at room temperature in a dark cupboard. The absorbance was then monitored after 30 min and converted into percentage of scavenging activity using the following formula:
% scavenging activity = [(Absorbance of control - Absorbance of test sample)/Absorbance of control]*100.
The experiments were carried out in triplicate and the percentages of DPPHs scavenged by the test samples were compared to that of vitamin C. These radical scavenging percentages were plotted against the logarithmic values of the concentrations, of extract and fractions, and a linear regression curve was established in order to calculate the RSa50 values, which are the amounts of sample necessary to decrease by 50% the free radical DPPH [21].

Anti-diarrheal studies

Infectious diarrhea

Diarrhoea Induction

A *Shigella flexneri* suspension was prepared at about 4 Mc Farland turbidity scale. For this, bacterial suspension was prepared from an 18 hrs old overnight Shigella culture on nutrient agar (NA). One millilitre (1ml) of this suspension containing about 12 x 10^8 colonies was orally administered to each animal. Only infected animals were selected and used [22].

Grouping of animals: Infected adult male rats (to avoid sex-dependent factors) were selected and divided into five groups (n = 5). Animals in groups I, II and III received 36.92, 73.85 and 147.65 mg/kg (corresponding to MIC/2, MIC and 2 x MIC respectively) body weight (BW) of ethyl acetate fraction, respectively (Figure 1). The fraction was first dissolved in DMSO (10% v/v). Group IV received ciprofloxacin (5 mg/kg) and served as positive control while group V received DMSO (10% v/v) as negative control. Treatment was done by administering 1 ml of ethyl acetate fraction orally every morning, for six days. Food and water were given to the animals ad libitum. Fecal samples were collected each day, just before administration of the treatment.

Assessment of stool bacterial density

The extent to which the animals complied with treatment was studied by quantifying the number of bacterial colonies in the fecal samples every day. Briefly, fecal samples were collected aseptically; 0.10 g was completely dissolved in 5 ml of autoclaved physiological saline water. 60 microliters (µL) of the resulting solution was transferred on a 90 mm Petri dishes containing solidified SS agar. After 24 hours of incubation at 37 °C, the number of Shigella flexneri colonies in each Petri dish was counted and the results were converted into the number of colonies per gram of faces per animal. Weight and appearance of feces; food and water intakes were also recorded every day during the experiment. After 6 days of treatment, animals were subjected to a 24 hrs fasting. These animals were sacrificed using chloroform vapors anaesthesia before dissecting. Blood samples from each animal were collected by cardiac puncture and analyzed for the determination of the hematocrit percentage. To measure the effect of the diarrhea on the concentration of blood elements, the concentrations of serum ions which are sodium and potassium were also measured using a spectrophotometer of atomic absorption.

Castor oil-induced diarrhea test

The effect of ethyl acetate fraction on diarrhoea was evaluated in rats using the castor oil-induced diarrhea method [20, 23]. Adult male rats were selected and divided into five groups (n = 5). Animals in groups I, II and III received orally 200, 400 and 600 mg/kg BW of ethyl acetate fraction respectively. Contrary to the infectious diarrhoea where dosages were calculated according to the antibacterial activity, the dosages used here were bases on previous studies as castor-oil induced diarrhea is a functional diarrhea and does not deal with infectious agents [22]. The ethyl acetate fraction was first of all dissolved in DMSO (10% v/v). Group IV received Imodium (2, 5 mg/kg) and served as positive control while group V received DMSO (10% v/v) and served as negative control. One hour after administration of the treatment, the animals received castor oil (1 ml/100g) orally and were individually placed in cages. The bottom of the cages was covered with a white sheet of paper for the observation of the number and consistency of faecal droppings. The number of both wet and dry droppings was counted every 30 min for 6 h and the white paper changed after each evaluation. The means of the stools passed by the treated groups were compared with that of the control.

Statistical Analysis

Statistical analyses were performed using SPSS software version. 19.0 (IBM, Armonk, NY, USA). The results of this experiment were expressed as the Mean ± Standard Deviation and compared using Waller-Duncan test. The results were statistically significant different for P values <0.05.

Results

Antibacterial activities

Table 1 and 2 shows the *in vitro* antibacterial activities of the crude extract and the fractions. From these Tables, it appears that crude extract and its fractions were found to be active with MIC values between 128 and 2048 µg/ml. These activities vary according to the bacterial strain. Ethyl acetate fraction figures as the most active, followed by residue and Hexane fraction, while the crude extract was less active, showing that the fractionation enhanced the antibacterial activity of all the fractions. After fractionation, antibacterial activity of each fraction was increased on certain strains and decreased on other strains (like showed in Table 4). It is also important to notice that crude extract and its fractions are bactericidal in 65 % of cases with MBC/MIC ≤ 4 [24, 25] and that only ethyl acetate fraction is bactericidal against all the bacterial strains. This later fraction also showed the lowest MIC value (128 µg/ml) on *Shigella flexneri* which is a bacteria strain responsible of diarrhea. It was then selected for the *in vivo* antidiarrheal activities.

Radical scavenging activities:

The DPPH radical scavenging activities of the crude extract and its fractions are summarized in Table 3. It appears that crude extract exhibited against DPPH a high antioxidant activity with the inhibitory concentration of the test samples scavenging 50 % of DPPH radical (RSa_{50}) between 2.09 and 5.36 µg/ml. These $RSsa_{50}$ values were all found to be higher than that of L-ascorbic acid (1.74

µg/ml). The study of the kinetics antioxidant activity showed that as L-ascorbic acid and some phytochemicals, methanol extract of *T. hirtum* and its fractions exert their scavenging activity against DPPH very promptly by stabilising the radical in the first five minutes following mixture.

Antidiarrheal activities

Infectious diarrhea

The Assessment of the treatment during the infectious diarrhea was done by counting the number of bacteria colonies in animal faeces after culture in SSA and the exponential raising observed before the beginning of the treatment indicated the infection was effective. Other signs typical to infectious or "invasive" diarrhea were observed. In fact rats developed signs as curling up, mold stools, bloody or mucus-linked lumpy feces with a fetid odor that likely expressed the presence of pus [22]. Fecal and frequency of stools also increased. During the second day of treatment, a progressive decreasing in the number of bacterial colonies in the feces of rats treated with the vegetal fraction and in the feces of those treated with ciprofloxacin was noticed. This decreasing was dose-dependent and bacteria decreases progressively in the feces of rats treated during the days of treatment and finally reduced to zero after 6 days of treatment, while bacterial the load was maintained at a high level (more than 2.10^5 colonies/g of feces). Few days post infection, a decrease in stools bacterial load of rats in the negative control group was observed.
No significant difference was observed in blood potassium concentration and hematocrit, but significant differences were also observed in serum concentration of sodium with higher values in rats receiving vegetal fraction. These values of sodium concentrations were proportional to the concentration of the fraction (Table 3)**.**

Castor oil-induced diarrhea

Ethyl acetate fraction inhibited significantly and almost completely the castor oil-induced diarrhea in rats at doses of 200 mg/kg and 400 mg/kg. This inhibition which was comparable to those the reference drug was observed on all the parameters measured. When looking at the weight gain and the frequency of stools, the latency period, water content of faeces, no significance difference was observed in rats of groups receiving imodium and those receiving vegetal fraction at dosage of 400 and 600 mg/kg. The smallest values of the latency period, volume of water consumed and weight gain were those observed for rats receiving DMSO 10 %, and these values were comparable ($P \leq 0,05$) to those obtained with rats receiving vegetal fraction at a dose of 200 mg/kg.

The ethyl acetate fraction of *T. hirtum* antidiarrheal activity against castor oil-induced diarrhea in rats. The vegetal fraction had a similar activity as Imodium, when tested at 400 and 600 mg/kg and statistically significantly reduced the frequency of defecation and the faecal droppings wetness when compared to untreated control rats.

Discussion

Antibacterial activities

The differences in bacteria susceptibility may be explained by differences in cell wall composition and/or genetic content of plasmids that can easily be transferred among microbial strains [26]. The differences between antibacterial activities of crude extract and fractions (Table 4) suggest that the large spectrum of activity observed was due to many antimicrobial groups rather than one. These differences observed for the same strain and different extracts could be explained by differences in the quality and quantity of active principles contained in the extracts or in the mechanisms by which they exert their antimicrobial action. It is also important to notice that crude extract and its fractions are bactericidal in 65 % of cases with MBC/MIC ≤ 4 [24, 25] and that only ethyl acetate fraction is bactericidal against all the bacterial strains. The antimicrobial activity of a phytochemical (crude extract) has been defined as significant when MIC is below 100 µg/mL, moderate when 100 µg/mL <MIC< 625 µg/mL or low when MIC>625 µg/mL [27, 28]. Therefore, the antibacterial results show that *Tristemma hirtum* extracts and mainly its ethyl acetate fraction could effectively be used for the treatment of a large range of antibacterial diseases.

Radical scavenging activities: The high scavenging activity *T. hirtum* extract could be explained by a variety of phenolic compounds (Table 4) which are potent antioxidant molecules. In fact, many studies had already proven the antioxidant activities of tannins, flavonoids, anthocyanins [20]. The high scavenging potential of these extract combined with their antibacterial effect constitute an important result because the extract could inhibit bacterial growth while stopping oxidative stress which is responsible of macromolecular degradation during infections. They could also be helpful in managing diverse disorders associated with oxidative stress like neurodegenerative diseases [29].

Antidiarrheal activities

The increase fecal and frequency of stools observed in infected animals could be due to intestinal fermentation caused by pH reduction [30, 31]. The progressive decreased in the number of bacterial colonies in the feces of rats treated with ethyl acetate fraction could be attributed to the ethyl acetate fraction of *T. hirtum* which may act in a dose-dependent manner killing bacteria through its bactericidal effect since bacteria decreases progressively in the feces of rats treated during treatment and finally reduced to zero after 6 days of treatment, while bacterial load was maintained at a high level (more than 2.10^5 colonies/g of feces). Action of the immune system could also be implicated since few days after infection, a low bacterial load in stools was observed in the negative control group.
The slight differences observed in sodium and potassium serum concentrations could be because during shigellosis, the germ invades intestinal epithelial cells and secretes a toxin (Verotoxin or VT) which inhibits intestinal absorption. Otherwise, differences in sodium serum concentration showed that during diarrheal episodes, this fraction could also contribute to physiological equilibrium by inhibiting sodium excretion which is the main extra cellular ion.

Castor oil causes diarrhea through its active metabolite, ricinoleic acid [32] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa [33, 34]. It also increases the volume of intestinal contents by preventing the reabsorption of

water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to the release of prostaglandin [35, 36]. Then, the ethyl acetate fraction which showed antidiarrheal activity comparable to that of immodium (when tested at 400 and 600 mg/kg) could exert its activity against castor oil-induced diarrhea by inhibiting prostaglandin synthesis or intestinal secretion [22, 37].

Antidiarrheal and antidysenteric activities of plants are due to tannins, alkaloids, flavonoids, steroids and terpenoids [38, 39]. Then therapeutic activity of ethyl acetate fraction against infectious and functional diarrhea could be explained by these groups of compounds which have been detected during phytochemical screening (Table 5). The high activity of the fraction (as the one of crude extract and the two other fractions) could be very helpful in the treatment of gastroenteritis because while eliminating the causative agent or inhibiting its effect, it could inhibit the intestinal oxidative processes which are very often responsible of symptoms of those diseases.

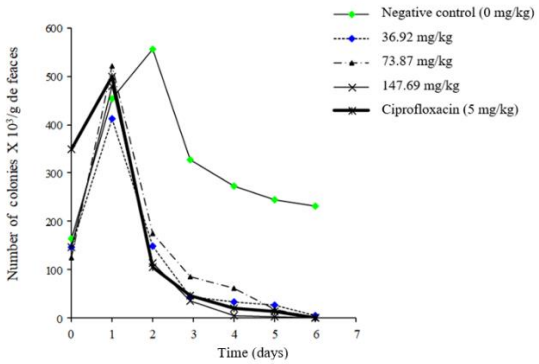

Figure 1. Bacterial charge evolution in the feces of rats infected according to different concentrations of ethyl acetate fraction of the methanol extract of *Tristemma hirtum*

Table 1. Inhibition parameters (MIC, MBC and MFC) of methanol crude extract of *Tristemma hirtum* and its fractions (µg/ml).

Test substances	Parameters*	Bacteria									
		Gram (+)								Gram (-)	
		PA	PM	SF	EC	KP	ST	SPA	SPB	SA	EF
Crude extract	CMI	2048	1024	256	256	256	512	256	256	1024	512
	CMB	2048	1024	8192	2048	4096	8192	2048	8192	8192	8192
	CMB/CMI	1	1	32	8	16	16	8	32	8	6
Hexane fraction	CMI	128	512	512	256	512	2048	512	1024	512	1024
	CMB	512	2048	8192	1024	2048	8192	2048	4096	4096	4096
	CMB/CMI	4	4	16	4	4	4	4	4	8	4
Ethyl acetate fraction	CMI	512	256	128	256	256	2048	256	512	512	512
	CMB	512	1024	512	1024	1024	8192	1024	512	1024	2048
	CMB/CMI	1	4	4	4	4	4	4	1	2	4
Residue fraction	CMI	128	512	256	256	512	512	256	512	512	1024
	CMB	512	2048	2048	1024	512	8192	1024	4096	4096	1024
	CMB/CMI	4	4	8	4	1	16	4	8	8	1
Ciprofloxacin	CMI	1	0,25	0,25	4	1	0,25	0,25	0,5	8	4
	CMB	1	1	0,5	4	1	2	0,5	2	8	16
	CMB/CMI	1	4	2	1	1	1	2	1	1	4

*MIC and MBC values in µg/ml PA: *Pseudomonas aeruginosa*, PM: *Proteus mirabilis*, SF: *Shigella flexneri*, EC: *Escherichia coli*, KP: *Klepsiella pneumonia*, ST: *Salmnella typhi*, SPA: *Salmnella paratyphi* A, SPB: *Salmnella paratyphiB*, SA: *Staphylococcus aureus,* EF: *Enterococcus faecalis*

Table2. DPPH radical scavenging activity of the methanol extract of *Tristemma hirtum* and its fractions: RSa_{50} values

Test substances	RSa_{50} (µg/ml)
Crude extract	5.33 ± 0.06^{c}
Hexane fraction	2.09 ± 0.01^{a}
Ethyl acetate fraction	3.27 ± 0.03^{b}
Residue fraction	3.66 ± 1.03^{b}
L-ascorbic acid	$1,74 \pm 0.01^{a}$

Values affected with different letters are significant different
RSa_{50}: Radical Scavenging activity 50 (The concentration of extract which scaveng 50%of DPPH free radical)

Table 3. Ions concentrations of Serum and percentage of hematocrit of rats infected according to different concentrations of ethyl acetate fraction of the methanol extract of *Tristemma hirtum*.

	Negative control	36.92 mg/kg	73.87 mg/kg	147.69 mg/kg	Ciprofloxacin (5 mg/kg)
Sodium	139.43 ± 6.67^{b}	140.58 ± 7.00^{a}	141.45 ± 3.50^{a}	144.51 ± 3.50^{a}	144.51 ± 3.49^{a}
Potassium	3.18 ± 0.00^{a}	4.31 ± 2.43^{a}	3.71 ± 0.92^{a}	4.78 ± 1.60^{a}	3.72 ± 1.84^{a}
Hematocrit (%)	40.33 ± 3.06^{a}	41.67 ± 5.50^{a}	39.67 ± 1.15^{a}	40.67 ± 3.06^{a}	39.00 ± 8.72^{a}

Values of the same column affected with different letters are significant different

Table 4. Parameters indicating the effect of ethyl acetate fraction of the methanol extract of *Tristemma hirtum* against castor oil induced diarrhea in according to dosage of ethyl acetate fraction of the methanol extract of *Tristemma hirtum*.

Doses (mg/kg)	**Latency period (min)**	**Frequency of feces**	**Weight of feces (g)**	**Water content of feces (ml)**	**Water consumption (ml)**	**Food consumption (g)**	**Percentage of body increase**
0	86.40 ± 5.00^{b}	8.80 ± 1.90^{a}	7.80 ± 0.84^{a}	3.18 ± 0.41^{a}	4.56 ± 1.25^{b}	4.56 ± 1.25^{a}	-0.14 ± 0.64^{a}
200	197.40± 8.64^{ab}	5.00 ± 1.00^{b}	5.02± 1.40^{b}	1.60 ± 0.17^{b}	5.01 ± 1.30^{b}	6.90 ± 1.37^{a}	-0.13 ± 0.63^{a}
400	234.40 ± 8.20^{a}	3.00± 0.67^{b}	3.50± 1.40^{c}	1.24 ± 0.25^{bc}	12.74 ± 1.27^{a}	12.74 ± 1.26^{b}	-0.06 ± 0.69^{b}
600	266.60 ± 42.45^{a}	3.60± 0.67^{b}	2.54± 0.78^{c}	1.00 ± 0.25^{c}	15.38 ± 3.69^{a}	15.38 ± 3.68^{b}	-0.038 ± 0.40^{b}
Imodium (2.5mg/kg)	270.00 ± 88.91^{a}	3.40 ± 0.55^{b}	2.40 ± 0.54^{c}	1.06 ± 0.88^{c}	14.62 ± 4.46^{a}	14.62 ± 4.45^{b}	-0.052 ± 0.56^{b}

Values of the same column affected with different letters are significant different

Table 5. Extraction yields, aspects and phytochemical composition of the plant extracts

Samples	**Yield * (%)**	**Physical aspect**	**Secondary metabolites**									
			alca	antho	anthra	couma	flavo	phen	sapo	ster	tan	triter
Crude extract	11.5	Dark green sticky	+	+	+	-	+	+	+	+	+	+
Hexane fraction	14.53	Dark creamy	-	+	-	-	-	-	-	+	-	-
Ethyl acetate fraction	05.87	Green steaky	+	+	-	-	+	+	-	+	+	+
Residue fraction	75.95	Brown powdery	+	+	+	-	+	+	+	+	+	-

- : absent; +: present; alca: alkaloids; antho:anthocyanins; anthra:anthraquinones; couma: coumarins; flavor: flavonoids; phen: phenols; sapo: saponins; ster: steroids; tan: tannins; triter: triterpens; *: yield of crude extract against weight of dry powder and yield of fractions against weight of crude extract

Conclusion

The results of the present studies provide clear evidence that the methanol extract of *T. hirtum* and its fractions possess antimicrobial and antioxidant activities. The ethyl acetate fraction appears as the fraction with the highest antibacterial activities and further presented antidiarrheal activities against functional and infectious diarrhea. All these results encourage further studies to isolate the active principles of the T. *hirtum* plant as well as its usage in the treatment of various diseases such as infectious diseases, diarrhea and the prevention of free radical related diseases.

Abbreviations

ATCC: American Type Culture Collection
BW: body weight
DMSO: dimethyl sulfoxide
DPPH*: 2,2 Diphenyl 1 picrylhydrazyl
HNC: Herbier National du Cameroun
MBC: Minimal bacteridal concentration
MeOH: Methanol
MHB: Mueller Hinton Broth
MHB: Mueller-Hinton Agar
MIC: Minimal inhibitory concentration
RSa50 Radical scavenging activity 50%
VT: Verotoxin

Authors' Contribution

JAKN, JRK and GNT designed the experiments and carried out the study, DED, JDT and JAKN wrote the manuscript; JRK supervised the work; all authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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