ANTIDIARRHEAL AND *IN VITRO* ANTIBACTERIAL ACTIVITIES OF LEAVES EXTRACTS OF *HIBISCUS ASPER*. HOOK. F. (MALVACEAE)

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ABSTRACT

Objective: To evaluate the antidiarrheal and antibacterial activities of aqueous and methanolic leaves extracts of *Hibiscus asper*.

Materials and Methods: The effect of aqueous and methanolic extracts of *H. asper* was assessing on the *in vitro* growth of 06 reference bacteria strains and 02 clinical isolates by determining the minimum inhibitory and bactericidal concentrations, using broth microdilution method as well as on the *Shigella flexneri* induced infectious diarrhea, castor oil-induced secretory diarrhea and magnesium sulfate-induced osmotic diarrhea models in rats.

Results: The methanolic extract was the most active, it inhibited the *in vitro* growth of 05 reference Gram-negative bacteria strains (*Escherichia coli* ATCC 11775, *E. coli* ATCC 8789, *E. coli* ATCC 10536, *Enterobacter aerogenes* ATCC 13048 and *Salmonella typhi* ATCC 6539), and one clinical isolate (*S. flexneri*). The minimum inhibitory concentrations values were between 512 and 1024 µg/ml. *In vivo*, methanolic and aqueous extracts, administered at the same dose (500 mg/kg) caused a significant decrease (p<0.05) in the bacteria load in the feces of rats, 8 and 12 days of treatment, respectively. The methanolic extract was the most active, it reduced bacteria load within a shorter duration of treatment (8 days). The results of this study indicate that the methanolic and aqueous extracts of leaves of *H. asper* after 6 hrs of observation, significantly inhibited (p<0.05; p<0.001) *in vivo*, diarrhea-induced experimentally by castor oil and magnesium sulfate, such as extending the latency, reducing the water content of feces, the frequency of defecation, and the number of wet defecations, compared to the negative control and to the dose 2.5 mg/kg of loperamide used as a reference substance.

Conclusion: We can, therefore, conclude that the leaves of *H. asper* possess antibacterial and antidiarrheal effects, resulting from their activity leading to the antibiotic mechanisms, the reabsorption of electrolytes (Na$^+$, K$^+$ and Cl$^-$) and water. These results reconcile the ethnopharmacological use of *H. asper* in the treatment of gastro-intestinal infections.

Keywords: Antibacterial, Antidiarrheal, *Hibiscus asper*.

INTRODUCTION

Diarrheal diseases are one of the leading causes of childhood morbidity and mortality in developing countries and are responsible for the death of millions of people each year. This diarrhea and the associated fecal urgency, in addition to incontinence, result from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hypermotility. These results in excess loss of fluid and electrolytes in feces [1]. It is an important health problem, especially in developing countries where it is hyper-endemic with parasitism. A variety of factors such as consumption of contaminated food water and reactions or intolerance to certain foods are implicated as causes. An estimated 1000 million episodes occur each year in children under 5 years of age leading to an estimated 5 million death in children fewer than 5 years of age/year [2]. Incidence of diarrheal diseases still remains high, despite intervention of government agencies and international organization to halt the trend. To combat this problem, the World Health Organization has initiated a diarrhea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches [3,4]. There is, therefore, an urgent need for the intensification of research into medicinal plant claim to be effective in the management of diarrheal diseases.

About 80% of individuals from emergent countries like Cameroon received traditional medicines including compounds derived from medicinal plants. Such medicinal plants can be exploited, since it has been shown that they are important sources of new chemical substances with potential therapeutic effects [5,6]. A number of medicinal plants has been used traditionally in the management of diarrheal diseases, and one of such medicinal plant is *Hibiscus asper*. It belongs to the family Malvaceae of the order Malvales represented by 250 species [7]. In the western region of Africa, this plant is widely used by traditional practitioners for the treatment of inflammation, anemia, jaundice, leucorrhea, poison antidote, depression, and dysmenorrhea [8,9]. In the western region of Cameroon, the leaves are highly recommended by tradipractitioners for the treatment of gastrointestinal disorders, abscesses, urethritis, and joint pain, but are also used as potent sedative, tonic and restorative as well as treating male infertility and skin infection [10]. In veterinary medicine, *H. asper* is used against the cutaneous infections of the domestic animals as well as an antiparasitic drug [8,11] demonstrated in previous work that the methanolic extract of *H. asper* leaves have antioxidant effects and improves neuroprotective effect against 6-hydroxydopamine-lesioned rat model of Parkinson's disease while [9] demonstrated that the same extract showed protective effect against Complete Freund-Adjuvant-induced arthritis. Phytochemical studies of *H. asper* revealed the presence of phenolic constituents and flavonoids which are known as potent antidiarrheal phytoconstituents [9]. The present study was prompted by the claim of some Cameroonian traditional health practitioners that maceration of *H. asper* leaves is an effective remedy for the treatment of diarrhea. Therefore, the scientific basis for the use of *H. asper* leaves as antidiarrheal remedy in traditional medicine is yet to be investigated. In furtherance of our search for potent medicinal agents from plant sources, we evaluated the antidiarrheal and *in vitro* antibacterial properties of the aqueous and methanolic extracts of *H. asper* leaves using experimental models of diarrhea in rats since

previous antidiarrheal works of other plants were based on ethical approved experimental animal induced-models, such as with castor oil, magnesium sulfate, and prostaglandine-E$_2$-induced-peristaltism/ enteropooling of the intestine; this for minimizing investigation concerning human.

MATERIALS AND METHODS

Plant material
H. asper leaves were collected in March 2011 in Bangou (West-region of Cameroon). The botanical identification of the plant was confirmed at the National Herbarium in Yaoundé (Cameroon) through a comparison with the voucher specimen Lucha034. The leaves were dried under shade, ground, and stored in an airtight container prior to extraction.

Extraction procedure
The aqueous extract was prepared by maceration of 100 g of powder in 1 L distilled water for 72 hr as indicated by the traditional healer. After filtration with filter paper (Whatman no 1), the filtrate was concentrated in a Selecta-25102 oven at 45°C, to give 20 g of the aqueous extract corresponding to an extraction yield of 20% (w/w). The other portion of leaf powder (100 g) was macerated in 1 L of methanol for 72 hr and the solvent removed from the extract under reduced pressure, using a Büchi (R-124) rotary evaporator at 65°C. This gave 22 g of the methanol extract, corresponding to a yield of 22% (w/w).

Microorganisms and growth conditions
The microorganisms used in this study consisted of six ATCC bacteria strains (*Enterococcus faecalis* ATCC 1054, *Escherichia coli* ATCC 11775, *E. coli* ATCC 8739, *E. coli* ATCC 10536, *Salmonella typhi* ATCC 6539, and *Enterobacter aerogenes* ATCC 13048), and two clinical isolates (*Staphylococcus aureus* and *Shigella flexneri*). The six bacteria strains and *S. flexneri* isolate were collected from "Centre Pasteur" (Yaoundé, Cameroun) while the second clinical isolate *S. aureus* was obtained from "Hôpital de district de Dschang" (Dschang, Cameroon). All bacteria used were grown at 35°C and maintained on nutrient Agar (Na, Conda, Madrid, Spain). Clinical trials ethical approval is currently granted by the ministry of public health (Cameroon) through National Ethics Committee.

Animals
Wistar albino rats weighing 100-140 g, of both sexes, were used for the antidiarrheal tests. They were bred in the animal house of the Department of Animal Biology, the University of Dschang, Cameroon under natural room conditions. Animals were fed with a standard diet and received water *ad libitum*. Prior to experimental protocol, the rats were acclimatized for 48 hr to laboratory conditions for minimizing any nonspecific stress. Experimental protocols used in this study were approved by the laboratory committee (Laboratory of Animal Physiology and Phytopharmacology, Department of Animal Biology, Faculty of Science, the University of Dschang-Cameroon) according to the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [12].

Drugs and chemicals
All reagents used in the study were of high purity. Ethylic Alcohol 95 (Geochim Cameroon), NaCl and dimethylsulfoxide (Sigma Chemicals Co. UK), iodonitrotetrazolium (INT) (Sigma, St. Louis), loperamide (Imodium), ciprofloxacin (Ciproma), Tween 80 (Roth), castor oil, and magnesium sulfate.

In vitro antibacterial activity
The *in vitro* antibacterial activity of the extract was performed by determining the minimum inhibitory concentrations (MICs) using broth microdilution method [12]. Briefly, the stock solution of *H. asper* extracts was dissolved in 8% dimethyl sulfoxide (DMSO)/Tween 80 and distilled water. Bacterial suspensions of about 1.5×10^8 CFU/ml (Mc Farland turbidity standard no 5) were prepared. To obtain the inoculate, these suspensions were diluted 100 times in Mueller Hinton broth to give

1.5×10^6 CFU/ml. The antimicrobial susceptibility tests were performed in 96 wells microplates. A serial two-fold dilution of the plant extracts was performed to obtain final concentration ranges from 1024 to 8 µg/ml for the extracts and from 64 to 0.5 µg/ml for the reference drug, in a total volume of 200 µl/well. Each well-contained the test substances at a particular concentration and 100 µl of the bacteria suspension (in Mueller Hinton broth). The plates were incubated at 37°C for 24 hrs. Microbial growth in each medium was determined colorimetrically, using INT. Viable bacteria changed the yellow dye of INT to a pink color. All concentrations at which no visible color changes were observed were considered as inhibitory concentrations, and the lowest of these concentrations was considered as the MIC. The bactericidal concentrations were determined by adding 50 µl aliquots of the preparations (without INT), which did not show any visible color change after incubation during MIC assays, into 150 µl of extract-free Mueller Hinton broth [12]. These preparations were further incubated at 37°C for 48 hrs, and bacteria growth was revealed by adding the INT as above. All extracts concentrations at which no color changes were observed were considered as bactericidal concentrations. The smallest of these concentrations was considered as the minimal bactericidal concentration. All samples were examined in triplicates. Ciprofloxacin was used as a positive control and 8% DMSO/ Tween 80 solution served as a negative control.

S. flexneri-induced diarrhea
Infectious diarrhea was induced in preweighed rats by oral administration of 2 ml of an inoculum 3×10^8 CFU/ml (the two McFarland standard), using a modified method developed by [13]. Prior to infections, rats were deprived from food for 18 hr, but received water *ad libitum*. Stools were also observed daily during 2 consecutive days before induction and cultivated for determining if there were any *S. flexneri* strains. Three-test doses (125, 250 and 500 mg/kg body weight [b.w]) of plant extracts were selected on a trial basis and administered orally by gavage to the animals of the last six groups. The fourth group (positive control) received ciprofloxacin at 2.5 mg/kg b.w as a reference drug. Groups 2 and 3 (negative controls) received distilled water and a mixture Tween 80/DMSO (8%), respectively. Group 1 (Neutral) was made of noninfected/ nontreated rats. Rats were weighed, and stools collected using a sterilized white cloth fixed under the grilling supporting the animals. They were observed and treated for 8 and 12 days (for the aqueous and methanol treated rats, respectively) from the day of induction. *S. flexneri* was enumerated in stool each 2 days and to achieve this, 0.5 g of diarrheal feces was homogenized in 5 ml sterile saline. Fifty µl of each tube were spread over the surface of Salmonella-Shigella (SS) agar petri dishes. Petri dishes were then incubated at 37°C for 24 hr, and the number of colonies was determined.

Castor oil-induced diarrhea in rats
Castor oil-induced diarrhea model was carried out using a modified method described by [14]. The animals were initially screened by observing stool's aspect. Those not showing diarrheic stools were selected for the final experiment. Fifty-four Wistar rats were randomly divided into nine equal groups (n=6) divided into controls, standard and test groups. The negative control groups received distilled water and Tween 80/DMSO (8%), respectively, (1 ml/100 g. b.w). The positive control group received loperamide at the dose of 2.5 mg/kg orally. The test groups received aqueous and methanolic extracts of *H. asper* leaves at doses of 125, 250, and 500 mg/kg orally. Each animal was placed in the individual cage, the floor of which was lined with filter paper, changed for every hour. Diarrhea was induced by oral administration of 1 ml/100 g. b.w. castor oil to each rat, 60 minutes after the above treatment. During the observation period of 6 hrs, the onset time, the frequency of defecation, the number of the wet spot, and the water content of feces were recorded. Water content of feces was expressed in term of percentages using the formula:

$$Wc(\%) = \left(\frac{Fw - Dw}{Fw} \right) \times 100$$

Wc = Water content of feces; Fw = Fresh weight (g); Dw = Dry weight (g).

Magnesium sulfate-induced diarrhea in rats

A similar protocol as for castor oil-induced diarrhea was followed. Diarrhea was induced by oral administration of magnesium sulfate at the dose of 2 g/kg to the animals, 60 minutes after administration of distilled water and Tween 80/DMSO (8%) to the negative control groups, loperamide (2.5 mg/kg) to the positive control group, the plant extracts in doses of 125, 250, and 500 mg/kg b.w to the test groups. All the treatments were administered orally.

Statistical analysis

The experimental results were expressed as the mean±standard deviation and mean±standard error of the mean. Data were evaluated by one-way analysis of variance and means were compared using Waller-Duncan and Tukey-Kramer post-tests at p<0.05 for infectious and metabolic (castor oil and magnesium sulphate) diarrhea, respectively.

RESULTS

Evaluation of antibacterial activity

The plant extracts had showed moderate activity against all the *E. coli* strains tested, *E. aerogenes, S. flexneri* and *S. typhi* (MIC values between 256 and 1024 µg/ml). The methanolic extract was the most active since it had showed antibacterial activity on six bacterial strains over the eight tested. The results are shown in Table 1.

***S. flexneri*-induced diarrhea in rats**

The aqueous and methanolic extracts of *H. asper* leaves, produced significant (p<0.05) dose-dependent decrease in the bacterial load in the feces of rats during 12 and 8 days of treatment, respectively. The dose 500 mg/kg of both extracts exhibited a highly significant (p<0.05) effect when compared to negative control group, with methanolic extract being the most active, since it reduced bacterial load in 8 days of treatment while the aqueous extract have done the same in 12 days (Figs. 1 and 2).

Castor oil-induced diarrhea in rats

The results obtained in the evaluation of the antidiarrheal activity of *H. asper* extracts in castor oil-induced diarrhea, showed that both aqueous and methanolic extracts at doses 500 and 125 mg/kg, respectively, highly significantly (p<0.001) prolonged the onset time, reduced the frequency of defecation, but not significantly (p>0.05) reduced the number of the wet spot and the water content of feces when compared to the negative control groups. Generally, the extracts inhibited diarrhea in a dose-dependent manner (Table 2, Figs. 3-10).

Table 1: Antibacterial activity (MIC, MBC) of aqueous and methanolic extracts of *H. asper*

Microorganisms	Parameters	Extracts (µg/ml)		Ciprofloxacin (µg/ml)
		EAHa	EMHa	
E. coli ATCC 11775	MIC	-	1024	>0.5
	MBC	-		64
E. coli ATCC 8739	MIC	1024	256	1
	MBC	-		2
E. coli ATCC 10536	MIC	512	256	1
	MBC	-	1024	2
E. faecalis	MIC	-		>0.5
	MBC	-		1
E. aerogenes	MIC	512	256	1
	MBC	-		2
S. flexneri	MIC	-	1024	0.5
	MBC	-		16
S. typhi	MIC	-	1024	1
	MBC	-		2
S. aureus	MIC	-		8
	MBC	-		2

-: MIC or MBC >1024 µg/ml, *E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, E. aerogenes: Enterobacter aerogenes, S. flexneri: Shigella flexneri, S. typhi: Salmonella typhi, S. aureus: Staphylococcus aureus*, MIC: Minimum inhibitory concentrations, MBC: Minimal bactericidal concentration, *H. asper: Hibiscus asper*

Magnesium sulphate-induced diarrhea in rats

All the doses of aqueous and methanolic extracts used, highly significantly (p<0.001) affected all the evaluated diarrheic parameters, in a dose-dependent manner. The inhibition was more effective in rats treated with a dose 500 mg/kg of both extracts, since it has significantly prolonged the onset time, reduced the defecation frequency, the water content of feces, and the number of wet spot, as compared to the negative control group and similarly to 2.5 mg/kg of loperamide used as a reference drug (Table 3, Figs. 11-18).
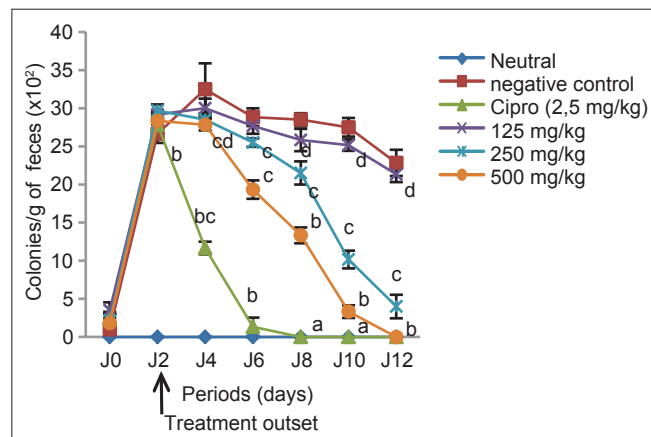

Fig. 1: Effect of aqueous extract on the fecal bacterial load in rats. Values are expressed as mean±standard deviation. a-d: p<0.05 when compared to the negative control
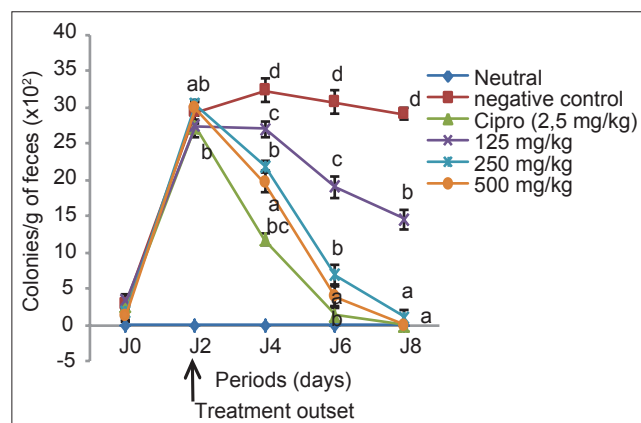

Fig. 2: Effect of methanolic extract on the fecal bacterial load in rats. Values are expressed asmean±standard deviation. a-d: p<0.05 when compared to the negative control
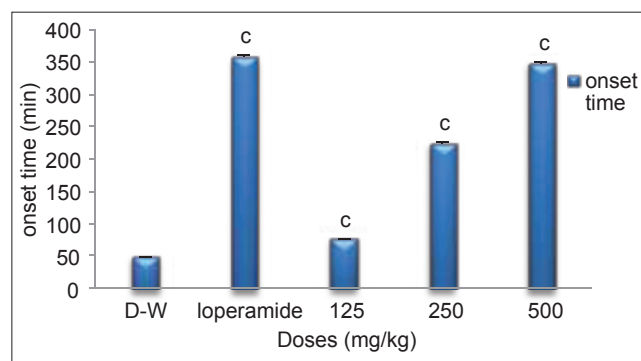

Fig. 3: Effect of the aqueous extract on the onset time in castor oil-induced diarrhea

Table 2: Antidiarrheal activity of *H. asper* extracts against castor oil-induced diarrhea

Treatment	Dose (mg/kg)	Onset (minutes)	Defecation frequency	Number of the wet spots	Water content of faeces (%)
Distilled water	1 ml/100 g b.w	49.00±0.36	02.33±0.33	02.00±0.51	82.08±0.49
Tween 80/DMSO 8%	1 ml/100 g b.w	55.00±1.50	02.17±0.31	02.00±0.52	62.25±3.89
Standard loperamide	2.5	358.50±1.50^{cγ}	0.83±0.30^{aα}	0.50±0.22	43.25±0.72^{c}
Aqueous extract	125	75.67±0.21^{c}	02.50±0.34	02.33±0.33	83.41±0.49
	250	224.50±0.22^{c}	02.33±0.33	01.33±0.42	76.75±0.70^{c}
	500	347.33±1.54^{c}	01.00±0.44	0.50±0.50	36.66±0.71^{c}
Methanolic extract	125	289.00±4.21^{γ}	0.83±0.31^{α}	0.83±0.31	56.33±0.86
	250	79.00±2.68^{γ}	02.50±0.50	02.50±0.50	90.88±1.72
	500	58.50±3.63	03.16±0.48	02.00±0.37	81.17±2.06

Values are mean±standard error of the mean. (n=6). For the same column, values affected by the same letter ($^{a}p<0.05$, $^{c}p<0.001$ when compared to distilled water and $^{α}p<0.05$, $^{γ}p<0.001$ when compared to Tween-DMSO) are not significantly different. DMSO: Dimethyl sulfoxide, *H. asper: Hibiscus asper*

Table 3: Antidiarrheal activity of *H. asper* extracts against magnesium sulfate-induced diarrhea

Treatment	Dose (mg/kg)	Onset (minutes)	Defecation frequency	Number of wet spot	Water content of feces (%)
Distilled water	1 ml/100 g b.w	178.00±0.73	03.66±0.21	03.16±0.16	67.46±1.30
Tween 80/DMSO 8%	1 ml/100 g b.w	262.50±45.52	03.66±0.21	03.33±0.21	82.62±1.81
Standard loperamide	2.5	360.00±0.00^{cα}	0.66±0.21^{cγ}	0.00±0.00^{cγ}	09.46±0.43^{cγ}
Aqueous extract	125	360.00±0.00^{c}	01.50±0.22^{c}	01.00±0.25^{c}	61.16±0.86^{c}
	250	360.00±0.00^{c}	01.33±0.21^{c}	0.50±0.22^{c}	54.95±0.49^{c}
	500	360.00±0.00^{c}	01.16±0.16^{c}	0.00±0.00^{c}	44.57±0.92^{c}
Methanolic extract	125	348.67±11.33	01.33±0.21^{γ}	0.66±0.21^{γ}	52.00±1.79^{γ}
	250	360.00±0.00^{α}	01.16±0.16^{γ}	0.50±0.22^{γ}	45.65±0.25^{γ}
	500	360.00±0.00^{α}	0.66±0.21^{γ}	0.00±0.00^{γ}	32.72±0.16^{γ}

Values are means±standard error of the mean. (n=6). For the same column, values affected by the same letter ($^{c}p<0.001$ when compared to distilled water and $^{α}p<0.05$, $^{γ}p<0.001$ when compared to Tween-DMSO) are not significantly different. DMSO: Dimethyl sulfoxide, *H. asper: Hibiscus asper*
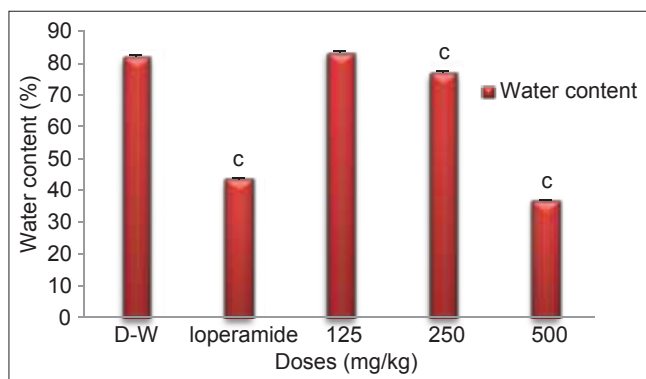

Fig. 4: Effect of the aqueous extract on the water content in castor oil-induced diarrhea
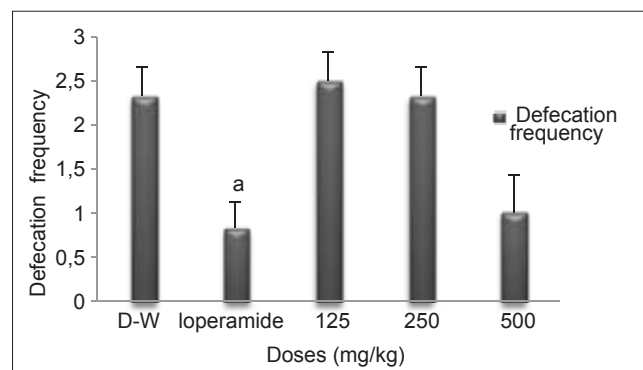

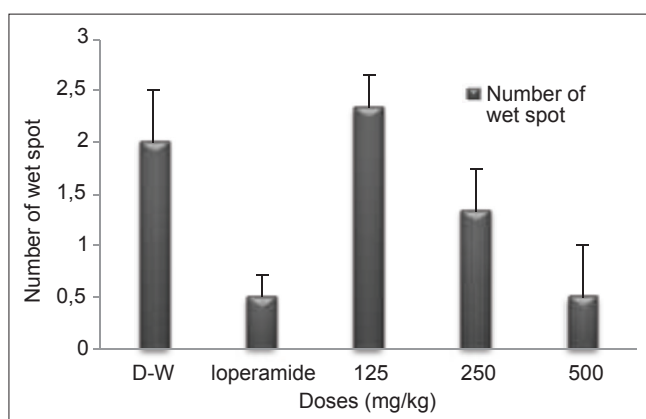**Fig. 6: Effect of the aqueous extract on the defecation frequency in castor oil-induced diarrhea**

Fig. 5: Effect of the aqueous extract on the number of wet spots in castor oil-induced diarrhea
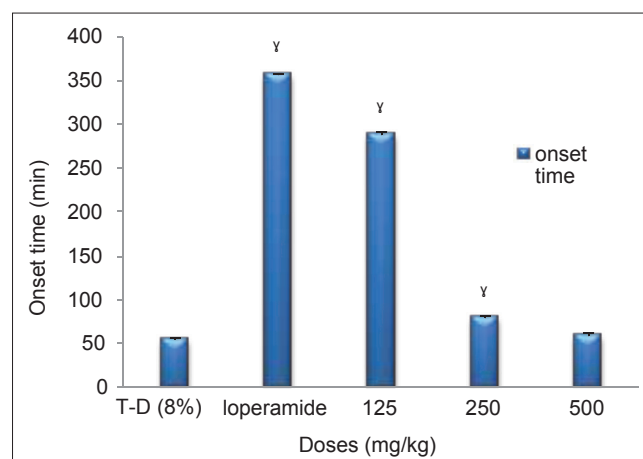

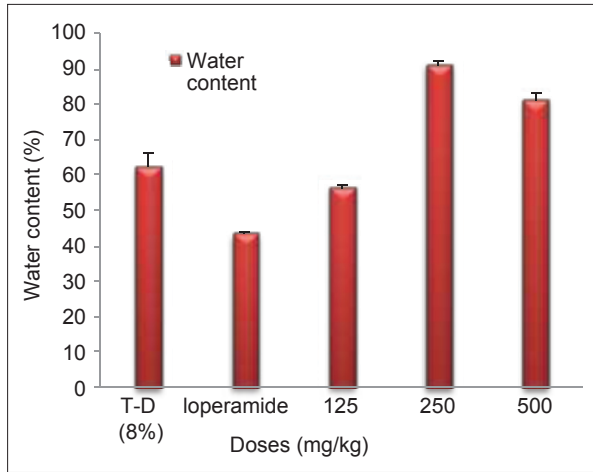**Fig 7: Effect of the methanolic extract on the onset time in castor oil-induced diarrhea**

Fig. 8: Effect of the methanolic extract on the water content in castor oil-induced diarrhea
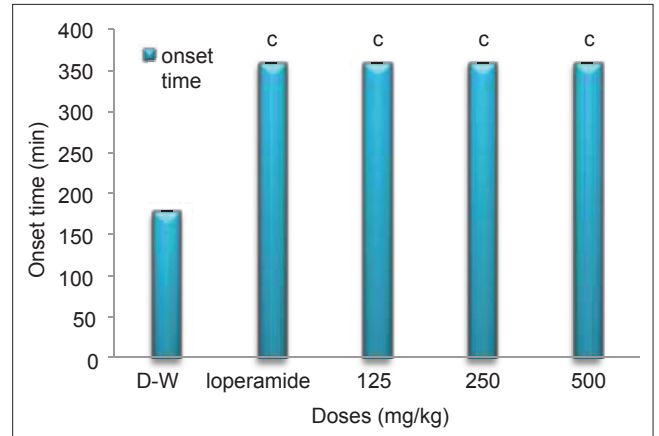

Fig. 11: Effect of the aqueous extract on the onset time in magnesium sulfate-induced diarrhea
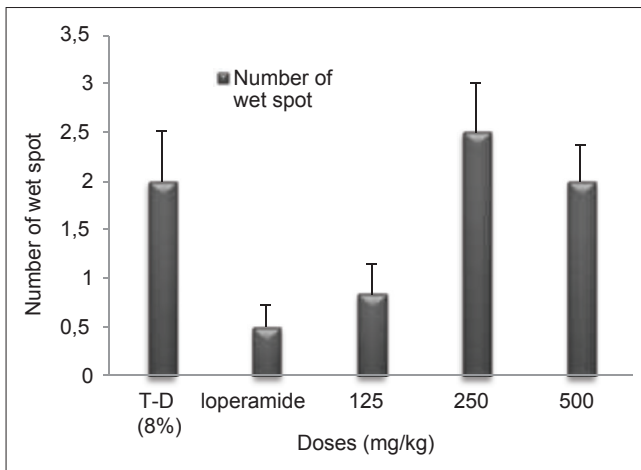

Fig. 9: Effect of the methanolic extract on the number of wet spot in castor oil-induced diarrhea
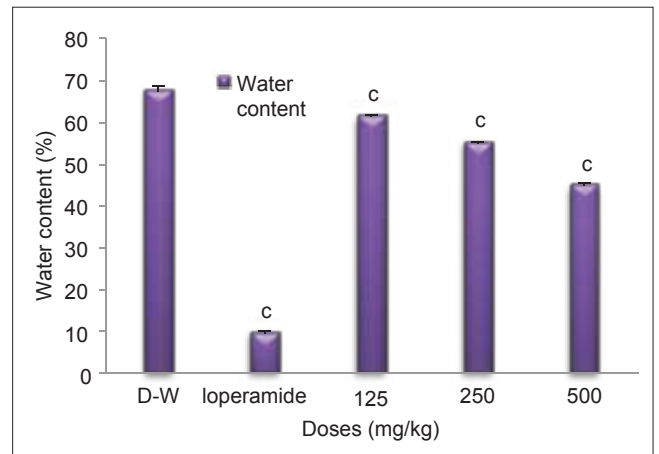

Fig.12: Effect of the aqueous extract on the water content in magnesium sulfate-induced diarrhea
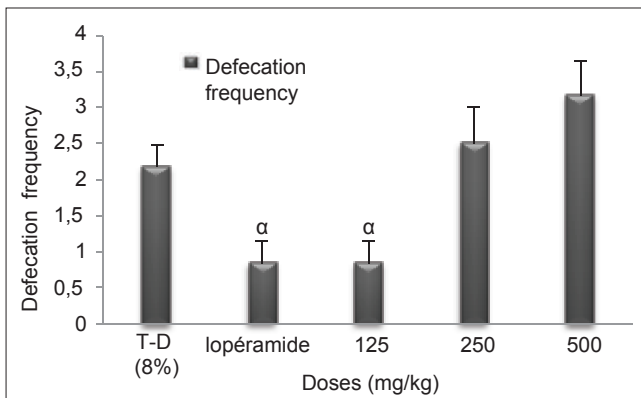

Fig. 10: Effect of the methanolic extract on the defecation frequency in castor oil-induced diarrhea
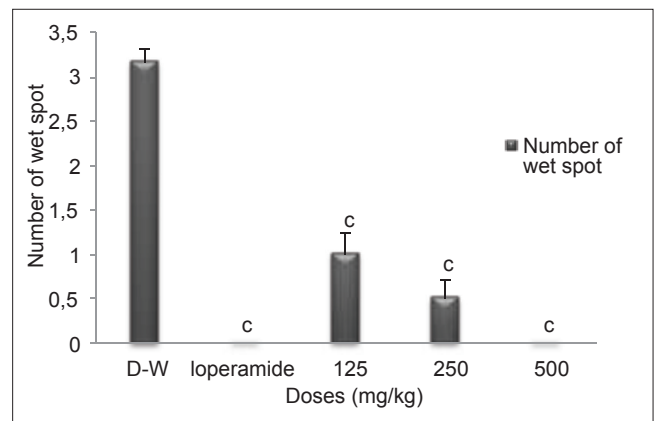

Figure 13: Effect of the aqueous extract on the number of wet spot in magnesium sulfate-induced diarrhea

DISCUSSION

The aim of this study was to evaluate the effect of aqueous and methanolic extracts of *H. asper* leaves on the *in vitro* growth of 06 reference enterobacterial strains and 02 clinical isolates as well as on the infectious, secretory, and osmotic-induced diarrhea in rats. The results indicate that both extracts developed moderate activities against bacterial strains tested. These activities can be linked to the presence of polyphenolic compounds and flavonoids identified by [9], which are secondary metabolites with potent antibacterial activity [15]. These metabolites have possibly acted through mechanisms which occurred at several levels of bacterial functioning. This includes rupture of the lipopolysaccharide membrane of Gram-negative bacteria such as *E. coli*, thus facilitating the restoration of protein channels which promoted the flow of the antimicrobial compounds to intrabacterial target sites or by inhibiting bacterial protein synthesis [16]. The results obtained at the end of the *in vitro* study led to the *in vivo* study on the model of diarrhea associated with
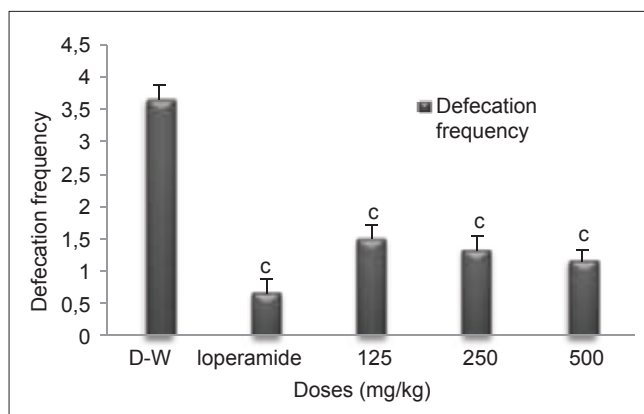
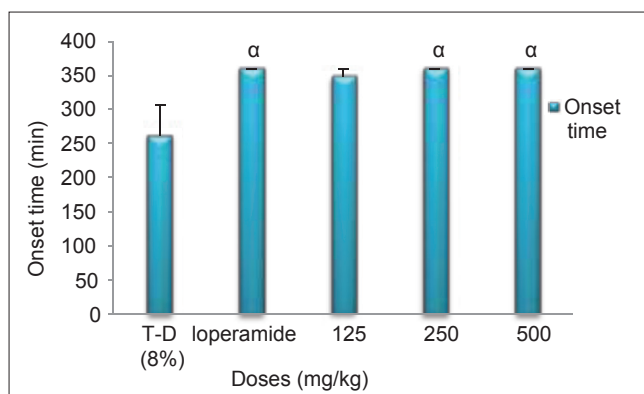**Fig. 14: Effect of the aqueous extract on defecation frequency in magnesium sulfate-induced-diarrhea**

Fig. 15: Effect of the methanolic extract on the onset time in magnesium sulfate-induced diarrhea
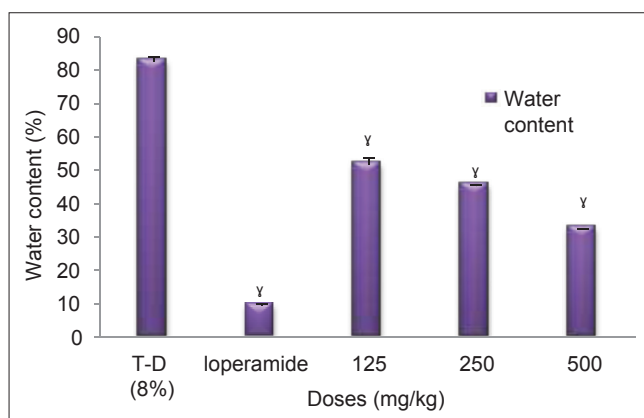

Fig. 16: Effect of the methanolic extract on water content in magnesium sulfate-induced diarrhea

Fig. 17: Effect of the methanolic extract on the number of wet spot in magnesium sulfate-induced diarrhea

Fig. 18: Effect of the methanolic extract on defecation frequency in magnesium sulfate-induced-diarrhea

bacterial infections. However, although it is only the methanolic extract that was active (MIC=1024 μl/ml) on *S. flexneri*, the choice of an induction of infectious diarrhea with this strain was based on the observations of [17], in which the *Shigella* species is the most important enteric cause of diarrhea. The installation of diarrhea on day 2 in rats, associated with morbidity observed in the latter could be explained by the intestinal invasion of *S. flexneri*, coupled with the action of its verotoxins. This bacteria would have penetrated the epithelial cells of the mucosa, where it quickly multiplied, resulting in the formation of abscesses and ulcers, which upset the mechanisms of intestinal reabsorption [18]. However, the decrease in bacterial load from the second day which followed the initiation of therapy in all infected and treated animals (with doses 125, 250 and
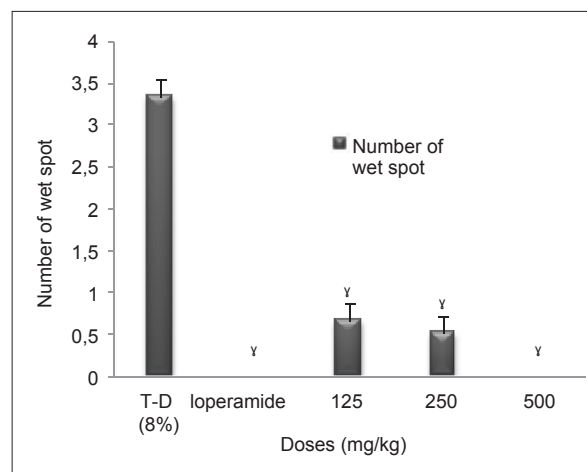
500 mg/kg), could be due to the presence of polyphenols and flavonoids in different extracts. These secondary metabolites have been reported to act on antibacterial mechanisms [19]. One might also think they would have boosted the immune system, such as stimulating the proliferation of lymphocytes [20]. Observed that the polyphenolic compounds and alkaloids are endowed with immunostimulatory properties. The fact that animals treated with different doses of methanolic extract recovered after a shorter period of treatment (8 days) than those that received the aqueous extract (12 days) could be due to the fact methanol has considerably ease the extraction of the active ingredients in the plant.

Secretory diarrhea is one of the most dangerous symptom of gastrointestinal disorders and is associated with an excessive defecation [21], that is why it became interesting to see if in addition to their action on infectious gastroenteritis, the extracts may have therapeutic activities against secretory diarrhea. The therapeutic properties of aqueous and methanolic extracts have been evaluated on the model of secretory diarrhea induced by castor oil. It is well-known that ricinoleic acid released after digestion by intestinal lipases of castor oil, leads to the establishment of an irritation, and inflammation of the mucosa. Degradation of the intestinal flora resulting, increase the process of biosynthesis of prostaglandins type E_2 (PgE_2) and histamine, causing the hypersecretion of electrolytes parallel to the increased of water [14,22,23].

The aqueous and methanolic extracts of the leaves of *H. asper* at doses of 500 and 125 mg/kg, respectively, may have acted through

antispasmodic and antisecretory mechanisms of polyphenols which inhibit the biosynthesis of PgE$_2$ and histamine as well as increasing the reabsorption of electrolytes (Na$^+$, K$^+$, Cl$^-$) [24] showed that polyphenols possess high anti-hypermotility and antihistamine power which could produce antidiarrheal effects. Loperamide works by reducing the rate of intestinal transit and increasing the capacity of fluid retention in the intestine [25]. The similarity between statistical values of these extracts and those of the dose 2.5 mg/kg of loperamide use as a reference drug, justified the activity of these same doses of aqueous and methanolic extracts on the reduction of the frequency and the water content of feces. In healthy individuals, too much magnesium can lead to osmotic diarrhea associated with distension of the bowel. These are observations that led to the evaluation of the activity of the extracts on a model of osmotic diarrhea induced by magnesium sulfate. It is known that magnesium sulfate causes increased secretion of electrolytes, creating a luminal osmotic imbalance [26]. The antidiarrheal activity of aqueous and methanolic extracts against the experimental osmotic diarrhea induced by magnesium sulfate could be attributed to their antisecretory actions simultaneously to restore the intestinal osmotic balance.

CONCLUSION

The aqueous and methanolic extracts of *H. asper* leaves possess significant antidiarrheal activity. Both extracts prolonged the onset time, significantly reduced the defecation frequency, the water content of feces and the number of the wet spots. Moreover, bacterial load in feces of pre-infected rats was significantly reduced. The methanolic extract inhibited the growth of human enterobacteria, showing an antibacterial activity. These findings demonstrate the effectiveness of leaves extracts of *H. asper*, as antidiarrheal and antibacterial agents.

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