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ORIGINAL ARTICLE

Anti-secretory effects of a dichloromethane fraction of the stem bark of *Piliostigma reticulatum* (Cesalpiniaceae)

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This study reports the effect of a dichloromethane fraction of the stem bark of *Piliostigma reticulatum,* a plant with anti-diarrhoeal properties, on the concentrations of electrolytes and the weight of water in castor oil-induced diarrhoea model in rats. The concentrations of ions in the supernatant of the small intestine content, obtained after centrifugation of the intraluminal fluid, were measured by flame photometry. The fraction showed a dose-dependent decrease of electrolytes concentration of [Na^+], [K^+], [Cl^-] and [Ca^{2+}], compared to the vehicle control. The ion concentrations were significantly reduced by the fraction at 125, 250 and 500 mg/kg, in the same range of inhibition obtained in rats treated by loperamide (5mg/kg), used as the reference anti-diarrhoeal drug. Quantity of water in faeces was also significantly reduced by the dichloromethane fraction at 250 and 500 mg/kg, and by loperamide. Results from the study showed that the dichloromethane fraction obtained from a crude extract of the stem bark of *P. reticulatum* possesses anti-secretory activity. These results suggest that the anti-diarrhoeal properties of the plant could partly be mediated by its anti-secretory activity and could therefore justify its use in traditional medicine to treat diarrhoea.

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Keywords*:* Castor oil induced-diarrhoea; electrolytes*;* loperamide, plant extract

INTRODUCTION

Diarrhoea is characterised by a discharge of semi-solid or watery faecal matter from the bowels three or more times per day (Hirchhorn, 1980; Snyder and Merson, 1982). It involves an increase in the fluidity and the number of faeces associated to an increased secretion of water and electrolytes (Field *et al.,* 1989; Longe and Dipiro, 1992; Dosso *et al.,* 2012). Diarrhoea is a public health problem especially for children under the age of five years. It is the second most common cause of infant deaths worldwide claiming over 2.6 million deaths in 2009 alone (UNICEF/WHO, 2009). It is estimated that 2.2 million children will die from diarrhoea and related diseases this year; 80% of them in the first two years of their life; 42,000 a week, 6,000 a day (Rehydration Project, 2012).

A report also indicates that up to 17% of children on admission in the paediatric ward die of diarrhoea (Mabeku *et al.,* 2006). In Côte d'Ivoire, the prevalence of diarrhoea in the population is 26.2%, and in Abidjan, the country's main city, it is evaluated to be 27.9% for diarrhoeas provoked by rotavirus in infants of 0-5 years old (Akoua-Koffi *et al,* 2007). Herbal medicine is a safe and economical source of bioactive compounds including substances of synergistic and/or side effects neutralizing potential (Gilani and Atta-ur-Rahman, 2005). It

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is now important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Harman *et al.,* 1992).

Piliostigma reticulatum (DC.) Horscht (Caesalpiniaceae) which is generally found in the west of Africa and particularly in the north of Côte d'Ivoire is traditionally used in treating many disorders, including diarrhoea (Yelemou *et al.,* 2007; Dosso *et al.,* 2012). Some of its vernacular names are niama (Malinké, Bambara), niamairi (Dioula) in Cote d'Ivoire (Kerharo and Bouquet, 1950), and abafe (Yoruba), kalga, kalgo (Hausa), thoingii pilostigma (local English) in Nigeria (Ainslie, 1937; Etuk *et al.,* 2009).

In a previous study, results showed that an ethanol extract of the stem bark of *Piliostigma reticulatum* significantly reduced the gastrointestinal transit, the number, volume and weight of faeces in rats (Dosso *et al.,* 2012). A preliminary investigation of various fractions obtained from the ethanolic extract of the stem bark of *Piliostigma reticulatum* suggests that the dichloromethane fraction bears highest anti-diarrhoeal properties (*unpublished data*). In the present study, we sought to investigate the anti-secretory activity, as a possible mechanism of action, of the dichloromethane fraction obtained from a crude ethanolic extract of the stem bark of *Piliostigma reticulatum* in a castor oil-induced diarrhoea model in rats.

MATERIALS AND METHODS

Plant collection

Stem barks of *Piliostigma reticulatum* (DC.) Horscht (Ceasalpiniaceae) were collected in Abidjan (South region of Côte d'Ivoire) in October 2007. The plant was identified and authenticated by Pr AKE-ASSI Laurent. A voucher specimen (N° 18033) of the plant was deposited in the herbarium of the National Centre of Floristic, University of Cocody-Abidjan.

Preparation of dichloromethane fraction

Stem barks of *Piliostigma reticulatum* were washed with water, cleaned, cut into smaller pieces and kept at room temperature for two weeks. They were then ground into a fine powder using a cutting mill (Retsch SM 100-1390 rev/min, Labo and Co, France). The powder (100 g) was extracted with 2 litres of a solution of ethanol (96%) / water (80:20, yielding a final ethanol concentration of 76.8%) for 24 hours with constant stirring using a shaking water bath (Kottermann, Germany) (this operation was repeated twice). The extract was filtered twice through cotton wool, then through a filter paper (Whatman grade 1, Sigma-Aldrich, France). The filtrate was concentrated using a rotavapor (Buchi, Switzerland) at 45°C, and dried on a water bath (Kottermann, Germany). The percentage yield was found to be 13.6%.

After successive liquid–liquid fractionations, five fractions (heptane, dichloromethane, ethyle acetate, butanol and water) were obtained from the crude ethanol extract (Harborn, 1984; Samsam-Shariat, 1992). From dried ethanol extract (starting with 10 g dissolved in 100 mL of water), heptane (800 mg = 8%), dichloromethane (900 mg = 9%), ethyl acetate (1700 mg = 17%), n-butanol (3200 mg = 32%) and aqueous (2100 mg = 21%) fractions were obtained respectively. The dichloromethane fraction was further selected for this study because in a previous preliminary study, it was the most potent anti -diarrhoeal agent (*unpublished data*). This was subsequently referred to as dicloromethane fraction or fraction.

Animals

Healthy, young adult albino rats of Wistar *strain* (age 5-6 weeks, weighing 150-200 g) of both sexes were obtained from UFR Biosciences (University of Cocody-Abidjan, Côte d'Ivoire). They were housed in stainless steel cages (34 cm ´ 47 cm ´ 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Ivograin®, Abidjan, Côte d'Ivoire) and given water *ad libitum*. They were allowed to acclimatize to standard laboratory temperature conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle) for one week before the experiments. They were deprived of food for at least 18 hours prior to experiments but allowed free access to drinking water. The equipment usage, handling and sacrificing of the animals were performed in accordance

with the European Council legislation 87/609/EEC for the protection of experimental animals (Mitjans, 2008). The protocols for the study were approved by the Departmental Ethics Committee.

Phytochemical analysis of the fraction

The dichloromethane fraction was screened for the presence of tannins, flavonoids, alkaloids, sterols, saponins, polyphenols, polyterpenes and anthraquinones. Detection of these constituents was performed according to the method described by Bekro *et al.,* (2007).

Castor oil-induced enteropooling and electrolyte secretion

Rats were divided into five groups of six animals each; they were pre-treated with normal saline (0.9% NaCl), loperamide (5 mg kg^{-1}) and dichloromethane fraction (125, 250 and 500 mg kg^{-1}) by oral gavage. After one hour, the rats received 2 ml of castor oil orally, and an hour later they were sacrificed. For each rat, the small intestine was removed and tied with thread at the pyloric end and the ileo-caecal junction. The intestinal content was drained into a graduated tube. The Na^+, K^+, Cl^- and Ca^{2+} concentrations in the supernatant, after centrifugation of the intraluminal fluid, were measured by flame photometry (Azdu *et al.,* 2003; Boominathan *et al.,* 2005).

Determination of the content of water in the faeces of rats

Thirty rats were divided into five groups of six animals each. The groups were pre-treated respectively with normal saline (0.9% NaCl), loperamide (5mg kg^{-1}) and dichloromethane fractions (125, 250 and 500 mg kg^{-1}) by oral administration gavage. After one hour, the rats received 2 ml of castor oil, and were sacrificed 1 h after castor oil administration. The small intestine was removed, tied with thread at the pyloric end and the ileo-caecal junction. The intestinal content was weighed with the electronic balance PM 4600® (Mettlertoledo, Germany) and dried under reduced pressure in a drying oven at 45° C (Memmert U30, Germany). According to the method of Navarro *et al.,* (2006) the difference between the weight of humid faeces (WHF) and the weight of dried faeces (WDF) was calculated to obtain the weight of water (WW). The percentage of intestinal content in water was also calculated.

$$\mathbf{WHF - WDF = WW}$$

$$\mathbf{\%\ of\ intestinal\ content\ in\ water} = \left(\frac{\mathbf{WW}}{\mathbf{WHF}}\right) \times \mathbf{100}$$

Data Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and IC_{50} determination. $P \leq 0.05$ was considered statistically significant in all analysis. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

RESULTS

Phytochemical analysis of the fraction

Phytochemical screening tests of dichloromethane fraction revealed the presence of major components such as tannins and flavonoids. Polyphenols and reducing sugars were also present, and anthraquinones, alkaloids, coumarins, polyterpenes and sterols were absent.

Effect of fraction on the concentration of sodium

The dichloromethane fraction dose-dependently and significantly ($P \leq 0.01$-0.001) decreased the concentration of sodium in comparison to the vehicle-treated group. This significant decrease was obtained at fraction doses of 250 and 500 mg mL^{-1} (Figure 1a). In rats treated by loperamide, the concentration of sodium was also significantly decreased by 46.48% ($P \leq 0.001$; Figure 1a; Table 1).

Effect of fraction on the concentration of potassium

The concentration of potassium was significantly reduced by the dichloromethane fraction at 125, 250 and 500 mg mL^{-1} to 0.79 ± 0.04; 0.49 ± 0.02 and 0.35 ± 0.03 mg mL^{-1} compared to the control

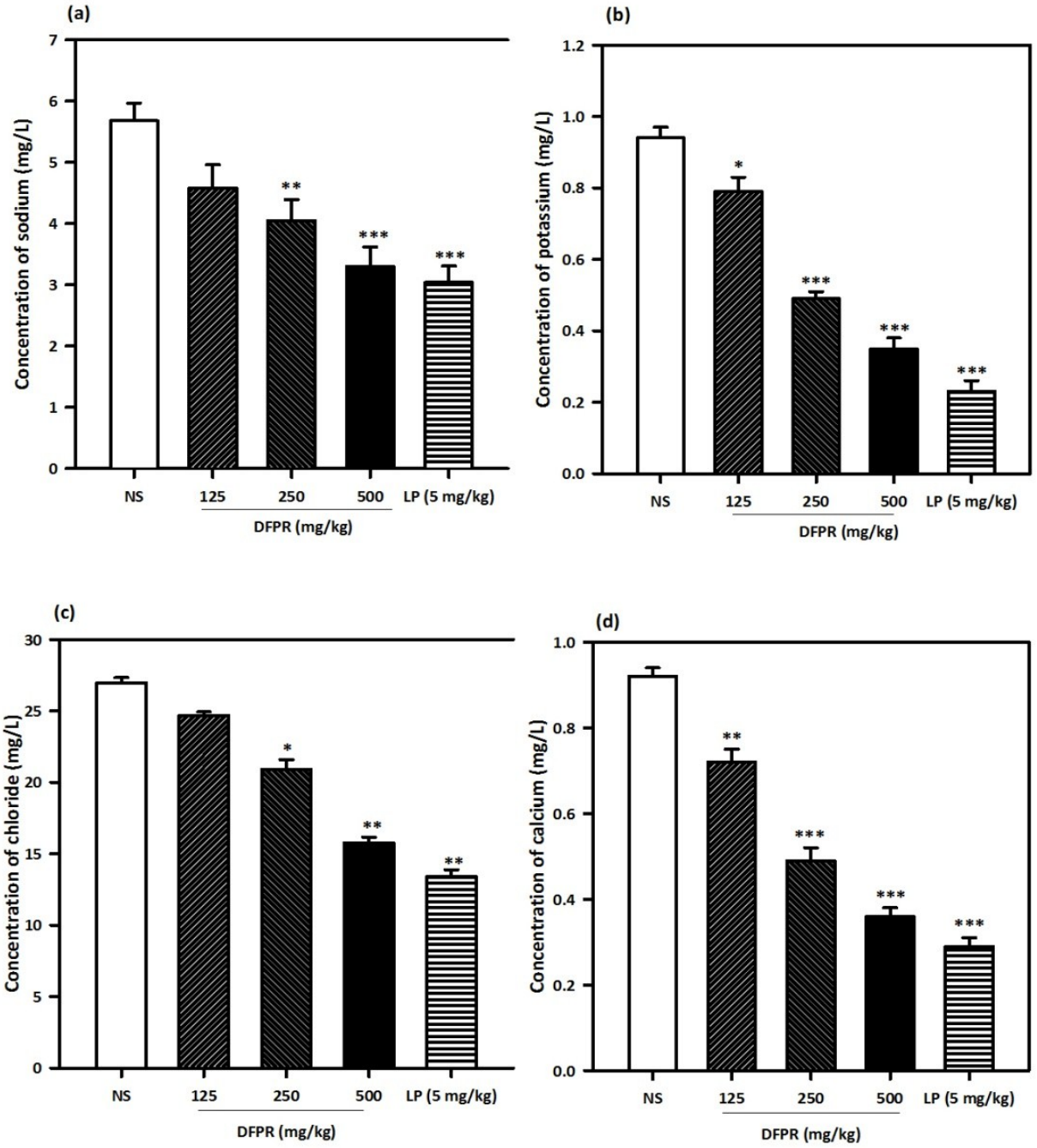

Figure 1: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on faecal concentration of (a) sodium; (b) potassium; (c) chloride and (d) calcium (mg/L). Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way ANOVA followed by a Dunnett's Multiple Comparison Test).

Table 1: The effect of the dichloromethane fraction and loperamide on the percent inhibition of electrolytes and content of water

Samples	Inhibition (%)					Content of water (%)
(mg kg^{-1})	Sodium	Potassium	Chloride	Calcium	Water	
NS	--	--	--	--	--	29.78
LP 5 mg kg^{-1}	46.48	75.53	50.29	68.48	61.90	50.95
DCMf 125	19.37	15.96	8.49	21.74	1.90	46.60
DCMf 250	28.70	47.87	22.40	46.74	29.05	41.73
DCMf 500	41.90	62.77	41.47	60.87	47.62	42.47

NS: Normal Saline; LP: Loperamide; DCMf: dichloromethane fraction

(0.94 ± 0.03 mg mL^{-1}) (P≤0.001) respectively (Figure 1b). The percentage of inhibition of the fraction at 500 mg mL^{-1} was 62.77% (Table 1). Loperamide also significantly reduced the concentration of the potassium to 0.23±0.03 mg mL^{-1} (P≤ 0.001) compared to the control.

Effect of fraction on the concentration of chloride

The decrease of the concentration of chloride was significant (P≤ 0.01; P≤ 0.001) at 250 and 500 mg mL^{-1} of fraction respectively (Figure 1c). The concentration of chloride was also significantly lowered by loperamide to 50.29% (P≤ 0.01) (Figure 1c; Table 1).

Effect of fraction on the concentration of calcium

The fraction significantly (P≤ 0.001) decreased the concentration of calcium to 0.72±0.03; 0.49±0.02 and 0.36 ± 0.02 mg mL^{-1}, at 125, 250 and 500 mg mL^{-1} respectively (Figure 1d). The percentages of inhibition of the fraction were 21.74, 46.74 and 60.87% respectively at 125, 250 and 500 mg mL^{-1} (Table 1). Loperamide also significantly reduced the concentration of calcium to 0.29±0.02 mg mL^{-1} (P≤ 0.001) (Figure 1d).

Effect of fraction on the weight of water

The weight of water in intestinal content was decreased by the dichloromethane fraction. The weight was significantly (P≤ 0.01) reduced at 250 and 500 mg mL^{-1} to 1.49±0.12 and 1.10±0.16 g. with percentage reductions of 29.05 and 47.62% respectively. Loperamide significantly decreased the weight of water to 0.80±0.20 g (P≤ 0.01) respectively (Figure 2).

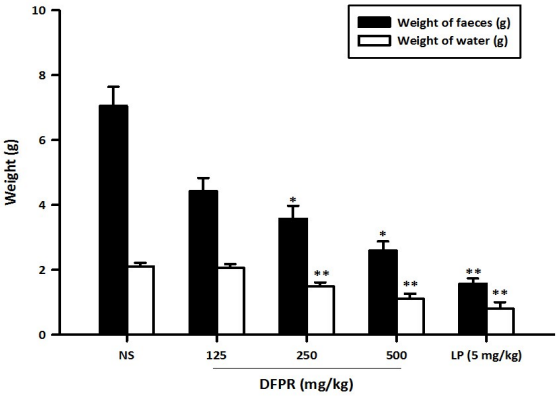

Figure 2: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on the weight of faeces and water contained in the faeces of rats. Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way ANOVA followed by a Dunnett's Multiple Comparison Test)

DISCUSSION

This study intended to demonstrate the anti-secretory activity of *Piliostigma reticulatum* in castor oil-induced diarrhoea in rats. Diarrhoea generally may be characterized as the abnormally frequent

expulsion of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines (George and Lutterodt, 1992; Gabriel *et al.,* 2004). Secretory and osmotic diarrhoea results in excessive loss of electrolytes and water (George and Lutterodt, 1992) leading to dehydration and subsequent death. WHO recommends oral rehydration solution which in many cases is a life saver (WHO, 2005). Castor oil causes diarrhoea due to its active metabolite, ricinolic acid (Ammon, 1974; Watson, 1962), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte and water permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez *et al.,* 1993). A previous study indicates an anti-diarrhoeal property of an ethanolic extract of the stem bark of *P. reticulatum* and that this activity is high in the dichloromethane fraction obtained from the ethanolic extract (Dosso *et al,.* 2012; *unpublished data*). Present results from this study suggest an added property since the fraction significantly decreased the concentration of the electrolytes and water content of faeces obtained from rats pre-treated with castor oil. This will go a long way as an adjunct treatment to oral rehydration therapy in the management of diarrhoea.

Loperamide, the reference agent used, has anti-motility and anti-secretory properties (Couper, 1987). The similarity of the results obtained by the fraction and the reference drug loperamide on the reduction of water quantity and ions concentrations could suggest the same mechanism-based on anti-mobility and anti-secretory properties of *P. reticulatum*.

The phytochemical screening of dichloromethane fraction of the stem bark of *P. reticulatum* revealed that tannins and flavonoids are the major components, whereas polyphenols and reducing sugars were minor components. It is possible that these components observed could be responsible for the anti-secretory activity of dichloromethane fraction of *P. reticulatum*.

CONCLUSION

This study demonstrates the anti-secretory property of dichloromethane fraction from the ethanolic extract of the stem bark of *P. reticulatum*. This may be responsible for its anti-diarrhoeal activity. This attribute provides a useful and additional rationale for the use of *P. reticulatum* in diarrhoea management by traditional healers.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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