



## Evaluation of anti-diarrhoeal properties of methanolic Root extract of *Piliostigma reticulatum* in rats

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### Abstract

*Piliostigma reticulatum* (DC) Hochst (Leguminosae) is an evergreen shrub reputed to possess a number of medicinal properties. The methanolic extract of *Piliostigma reticulatum* root (Leguminosae) was evaluated for anti-diarrhoeal activity in rats using castor oil-induced diarrhoea and fluid accumulation and activated charcoal test models. Phytochemical screening revealed the presence of tannins, glycosides, saponins, sterols, alkaloid and balsam. The extract (at doses of 100 and 200 mg/kg) and atropine (3 mg/kg) significantly inhibited castor oil-induced diarrhoea (53.5–72.1%) and fluid accumulation (35.17–71.03%) and small intestinal transit (9.38–21.74%) in the rats. The results obtained in this study revealed that the extract has remarkable anti-diarrhoeal effect, which may be due to the presence of the tannins, alkaloids and saponins constituents and can thus be used in the treatment of non-specific diarrhoea.

*Keywords: Piliostigma reticulatum, Anti-diarrhoeal activity, Rat, Gastrointestinal tract.*

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### Introduction

Diarrhoea is an important health problem worldwide especially in developing countries and accounts for more than 5–8 million deaths in infants and children under 5 years each year(1). It may be defined as an abnormal increase in the frequency and volume (more than 200 g/day) of soft or liquid stool.

The volume of stool is determined principally by the gastrointestinal fluid content, which accounts for 70–85% of total stool weight (2). The net stool fluid is a balance between luminal input and output. Net absorption of water occurs in the small intestine in response to osmotic gradients and can be altered by neuro-humoral mechanism, pathogens and drugs, which affect reabsorption and/or gastrointestinal motility.

Diarrhoea therefore results from an imbalance in absorptive and secretory mechanisms in the gastrointestinal tract resulting in an excessive loss of fluid in the faeces. Currently, there is renewed interest in herbal remedies for the treatment of

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many ailments. Medicinal plants are a promising source of anti-diarrhoeal agents. Several studies have evaluated the effectiveness of some traditional medicines used in the treatment of diarrhoea in different countries and continents (3).

The plant *Piliostigma reticulatum* (DC) Hochst is an evergreen shrub or small tree with a twisted bole widely distributed in Northern, Western and Eastern Africa. It is reported to possess medicinal properties similar to *Piliostigma thonningii* (4). Its bark extract is used for stomach pain, indigestion and as an astringent in the treatment of diarrhoea and dysentery (4).

Chemical compounds isolated from the leaves include quercetin and its derivatives (5). Intraperitoneal administration of quercetin has been shown to possess anti-diarrhoeal activity (6). The root is used in Northern Nigeria for the treatment of diarrhoea and is applied to the surface of wounds and ulcers.

The aim of this study is to evaluate the methanolic root extract of *Piliostigma reticulatum* for its anti-diarrhoeal activity in rats.

## Materials and methods

### *Plant Material*

Fresh roots of *Piliostigma reticulatum* were collected from Suleja, Niger State, Nigeria in February, 2006. It was identified by Mallam Muazzam of the Medicinal Plant Research and Traditional Medicine (MPR&TM) Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. A voucher specimen (NIPRD/H/6181) of the root has been deposited in Medicinal Plant Research & Traditional Medicine (MPR&TM) departmental herbarium.

### *Preparation of Extract*

The root of *P. reticulatum* was cleaned, air-dried and ground in a mortar to obtain a coarse powder material. Extraction was carried out by shaking 500 g of the powder in 2.5 litres of 70% methanol (a ratio of 1:5) and shaken using GFL Shaker (No. 3017 MBH, Germany) for 72 hours. The resultant mixture was then filtered in vacuum and concentrated using a rotary evaporator at temperature not exceeding 40°C. Methanol was evaporated by heating the concentrate over a water bath to give a solvent-free extract. Aliquot portions of the dried plant extracts were freshly reconstituted with distilled water to obtain the desired concentration for each study.

### *Animals*

Adult male and female wistar rats weighing 150–350 g obtained from the Animal Facility Centre (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD) were used. They were housed in stainless steel cages at room temperature with 12/12 h light-dark cycle, fed on standard rat feed and given water *ad libitum*. Prior to each experiment, the rats were starved for 24 hours, but

allowed free access to water. Each experimental group consisted of five rats housed in separate cages.

#### *Drugs*

Castor oil (Bell Sons & Co., England), Tragacanth powder (Bush, Boake Allen, England) and Atropine (Sigma Chemical Co., USA) were used as standard agents.

#### *Phytochemical Tests*

Preliminary phytochemical screening of the powdered plant material for the presence of different chemical constituents were carried out using standard test procedure (7). Secondary metabolites tested for included carbohydrates, free reducing sugars, combined reducing sugars, tannins, free anthraquinone glycosides, reduced anthraquinone glycosides, saponins, sterols, balsams, alkaloids, monosaccharides, flavonoids, terpenes and resins.

#### *Pharmacological Studies*

##### *Effect on castor oil-induced diarrhoea*

This study was carried out as described by Awouters *et al* (8) and Mukherjee *et al* (9). Five groups of five rats each (n = 5) were used. Group I served as the control and was given 1 ml normal saline/kg, group II received 3 mg/kg atropine intraperitoneally (i.p.), while groups III, IV and V received 50, 100, 200 mg extract/kg i.p. respectively. One ml of castor oil was given to each rat orally 1 hour later and each animal was then placed in an observation cage with a sheet of white paper. The number of both formed (dry) and wet stools passed by the rat were counted every hour for a period of 4 hours. Mean of the stools passed by the treated groups were compared with that of the control (normal saline) group.

##### *Effect on castor oil-induced intestinal fluid accumulation*

This study was carried out as described by Adzu *et al* (10). Five groups of five rats each were used and treated as follows: group I (control) received 10 ml normal saline/kg extract or atropine as described in 2.6.1. After 1 hour, 1 ml castor oil was administered to each rat. Thirty minutes later, the rats were killed by chloroform inhalation. Regions of the gastrointestinal tract extending from the pylorus to the ileo-caecal junction were dissected out. The contents were expelled into a petri dish drawn into a 5 ml syringe and the volumes measured.

##### *Gastrointestinal transit test*

The method described by Capasso *et al* (11) was adopted. Rats were randomized into groups of five rats each. Group I (control) was treated with the vehicle (10% aqueous tragacanth), groups II, III, IV received 50, 100 and 200 mg extract/kg orally respectively, while group V received 0.1 mg/kg atropine intraperitoneally. After 30 minutes, each rat was given 1 ml of 5% deactivated charcoal suspended in

10% aqueous tragacanth powder orally. The rats were sacrificed 30 minutes later by chloroform inhalation the abdomen opened, the small intestine removed and immersed in normal saline to halt further propulsive activity. The distance travelled by the charcoal plug from the pylorus to caecum was then measured.

#### *Data Analysis*

All the results were expressed as mean  $\pm$  standard error of mean (SEM) and simple percentages. Student's t-test was used to analyze the result between groups with  $P < 0.05$  taken as level of significance in all cases.

### **Results**

#### *Phytochemical Tests*

Qualitative phytochemical analysis of the root extract gave positive reactions for the following secondary metabolites: carbohydrates, free reducing sugars, combined reducing sugars, tannins, free anthraquinone glycosides, reduced anthraquinone glycosides, saponins, sterols, balsams and alkaloids. Monosaccharides, flavonoids, terpenes and resins were however absent.

#### *Castor Oil-induced Diarrhoea*

Thirty minutes after administration of castor oil, the diarrhoea was clinically apparent in all the rats in the control group. The extract caused a dose-dependent (14.0-61.9%) inhibition of the severity of diarrhoea induced by castor oil. At 50 and 100 mg extract/kg, significant ( $P < 0.05$ ) reduction in diarrhoea (53.5 and 62.8% respectively) was observed. Atropine also inhibited (72.1%) castor oil-induced diarrhoea significantly (Table 1).

#### *Castor Oil-induced Intestinal Fluid Accumulation*

Table 1: Effect of methanolic root extract of *P. reticulatum* on castor oil -induced diarrhoea in rats

<i>Group</i>	<i>Total Frequency of Diarrhoea</i>	<i>Mean ES <math>\pm</math> SEM (Mean Frequency of Diarrhoea)</i>	<i>% Inhibition</i>
Control	43	8.6 $\pm$ 1.7	-
Extract 50 mg/kg	35	7.0 $\pm$ 1.86	18.6
Extract 100 mg/kg	20	4.0 $\pm$ 1.025*	53.5
Extract 200 mg/kg	16	3.2 $\pm$ 0.79*	62.8
Atropine 3 mg/kg	12	2.4 $\pm$ 1.40*	72.1

\* Significantly different from the control at  $P < 0.05$

The extract suppressed intestinal fluid accumulation at all doses used but significantly at 100 (55.86%) and 200 (35.17%) mg extract/kg. The effect was however not dose-dependent. Atropine was observed to be more potent than the extract in inhibiting the castor oil-induced intestinal fluid accumulation (Table 2).

#### *Gastrointestinal Transit Test*

Table 2: Effect of methanolic root extract of *P. reticulatum* on castor oil -induced intestinal fluid accumulation in rats

<i>Group</i>	<i>Fluid Volume (ml)</i>	<i>% Inhibition</i>
Normal saline	1.45 ± 0.17	-
Extract 50 mg/kg	1.15 ± 0.60	20.68
Extract 100 mg/kg	0.64 ± 0.11*	55.86
Extract 200 mg/kg	0.94 ± 0.13*	35.17
Atropine 3 mg/kg	0.42 ± 0.11*	71.03

\* Significantly different from the control at P < 0.05.

The percentage intestinal transit was significantly reduced at 100 and 200 mg extract/kg used and much more markedly by atropine (9.38%). This effect was also dose-dependent and was more effective at 100 mg extract/kg body weight in the rats (Table 3).

Table 3: Effect of methanolic root extract of *P. reticulatum* on gastrointestinal transit test in rats

<i>Group</i>	<i>Mean Intestinal Length (cm)</i>	<i>Mean Distance Travelled by Charcoal</i>	<i>% Inhibition</i>
Control	76.8 ± 4.28	60.58 ± 4.10	-
Extract 50 mg/kg	105.0 ± 3.18	58.2 ± 2.31	3.92
Extract 100 mg/kg	90.40 ± 7.16	13.40 ± 0.30*	77.88
Extract 200 mg/kg	101.2 ± 37.14	22.00 ± 1.14*	63.68
Atropine 0.1 mg/kg	106.6 ± 8.89	8.0 ± 0.71*	86.79

\* Significantly different from the control at P < 0.05

## Discussion

The qualitative phytochemical test revealed the presence of tannins, glycosides, saponins, alkaloids and balsam in the methanolic extract of *P. reticulatum*. Anti-diarrhoeal and anti-dysenteric properties of medicinal plants have been reported to be due to tannins (12), alkaloids (13), saponins, reducing sugars, sterols and triterpenes. These substances may precipitate proteins of the enterocytes, reduce peristaltic movement and intestinal activity(12). Protein tannates make the intestinal mucosa more resistant and reduce its secretion.

Many anti-diarrhoeals act by reducing the gastrointestinal motility and/or secretions. The inhibition of experimentally-induced diarrhoea and reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential anti-diarrhoeal agent.

Lipolysis of castor oil in small intestine releases ricinoleic acid, the active principle that causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins which stimulate motility and secretion (14, 15). Inhibitors of prostaglandin biosynthesis have been observed to delay castor oil-induced diarrhoea (8). It has also been shown that E-type of prostaglandins cause diarrhoea in experimental animals as well as in human beings. Castor oil increases the volume of intestine mass by preventing the reabsorption of electrolyte (sodium chloride) and water resulting in fluid accumulation in the lumen. Drugs affecting motility, frequency and consistency of diarrhoea have been shown to also affect secretion (6).

This study showed that the methanolic extract of *P. reticulatum*, reduced the severity and frequency of castor oil-induced diarrhoea and significantly inhibited castor oil-induced fluid accumulation and the volume of intestinal content. In addition, the extract at doses of 100 and 200 mg/kg significantly reduced the intestinal transit as observed in the charcoal meal test. Slowing of gastrointestinal motility prolongs transit time of faecal mass and allows more time for the absorption of water and electrolytes from the intestinal lumen.

Atropine, an anti-muscarinic drug, produced significant reduction in severity and frequency of castor oil-induced diarrhoea, and intestinal fluid accumulation as well as intestinal transit. Generally, muscarinic receptor antagonists reduce tone, motility and secretions in the gastrointestinal tract suggesting the probable involvement of anti-muscarinic receptor mechanism in the anti-diarrhoeal activity of the extract.

The results of this study showed that methanolic extract of *P. reticulatum* contains active principles with significant anti-diarrhoeal properties and scientifically justify its use in the treatment of non-specific (secretory and functional) diarrhoea. Further studies will be carried out to establish the safety of the extract and isolate the active principle(s) responsible for its anti-diarrhoeal effect.

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