



Original Paper

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Anti-diarrhoeal activity of the methanolic leaf extract of *Phyllanthus muellerianus*

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ABSTRACT

The methanolic leaf extract of *Phyllanthus muellerianus* was investigated for anti-diarrhoeal activity. The anti-diarrhoeal activities were investigated using the castor oil-induced diarrhoea, magnesium sulphate-induced diarrhoea, small intestinal and distal colonic propulsion, isolated rabbit jejunum and castor oil-induced intestinal fluid accumulation. The results revealed that the methanolic leaf extract of *Phyllanthus muellerianus* significantly ($p < 0.05$) and dose-dependently inhibited castor oil-induced diarrhoea, magnesium sulphate-induced diarrhoea, and also inhibited small intestinal propulsion and distal colonic propulsion. The extract inhibited the spontaneous movement of the isolated rabbit jejunum and reduced castor oil-induced intestinal fluid accumulation. The intraperitoneal LD₅₀ of the extract in mice was found to be 547.7 mg/kg and preliminary phytochemical screening revealed the presence of tannins, carbohydrates, free anthraquinones and flavonoids. The results of this study indicate the presence of biologically active substance(s) which may be beneficial in the management of diarrhoea.

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Key words: *Phyllanthus muellerianus*, Euphobiaceae, Herbal medicine, Anti-diarrhoea.

INTRODUCTION

Diarrhoea is an intestinal disorder characterized by passage of loose watery stools, more frequent than is normal for the individual; the overall weight and volume of the stool is increased (more than 200g or ml/day), and the water content is increased to 60% – 90% (Hirchhorn, 1980; Fuller et al., 2004). It is usually caused by substances that increase the osmotic influx of water and ion to the intestinal lumen (Velazquez et al., 2006). The disease still remains a major cause of morbidity and mortality in developing countries with about four billion cases occurring each year, and in developing countries such as sub-Saharan Africa, children may experience between 3 – 5 episodes each

year (Farthing, 2006), causing about 17% of under-five death plus 3% of neonatal death (Bryce et al., 2005).

The plant *phyllanthus muellerianus* Arg. (Euphobiaceae) is a perennial plant found mostly in the forest area in tropical region of West Africa, known in Hausa land as ‘majiriyar kurumi’ where the leaves have an age long usage as remedy for treating gastrointestinal disorders (Dalziel, 1937). The leaves boiled with palm fruits are also given to women after delivery; an infusion of the leaves and roots is given for eruptive fevers in children, the fresh pounded leaves are applied to wounds and the fresh juice of the leaves used as a remedy for eye problems and decoction of the leaves and young twigs is

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given for chest complains, to relieve urethral discharges and for dysentery (Dalziel, 1937). The plant has been reported to have antimicrobial (Onocha et al., 2003), anti-plasmodial (Zihiri et al., 2005), analgesic, anti-inflammatory and sedative properties (Anuka et al., 2005). However, little information was reported in literature about the effects of *P. muellerianus* on gastrointestinal activities. This work was designed to determine the anti-diarrhoeal activity of the methanol extract of the plant.

MATERIALS AND METHODS

Plant material

The leaves of *Phyllanthus muellerianus* were collected in the month of May 2007 around Idu, FCT-Abuja, Nigeria. The plant was identified and authenticated by Jamilah Ibrahim and Ibrahim Muazzam of the Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. Voucher specimen no. NIPRD 11/5559 was prepared and deposited at the NIPRD herbarium for future reference.

Extraction

The fresh leaves of *Phyllanthus muellerianus* were air-dried and pounded into powder using mortar and pestle. 200 g of the powdered leaves were macerated in 500 ml of methanol for 24 h with occasional shaking. This was filtered and the filtrate dried over a water bath maintained at 80 °C to give a solid with a mean yield of 6.25% w/w.

Animals

All experiments performed on laboratory animals in this study followed the 'Principles of Laboratory animal care' (NIH publication No. 85 – 23, revised (1985). Swiss albino mice (25 – 30 g) of both sexes, Wistar rats (180 – 200 g), and New Zealand rabbits (1.5 – 2.5 kg) bred at uniform condition at the Animal Facility Centre, NIPRD, were used in this study. They were housed in standard polypropylene cages with saw dust as

beddings, under standard condition of temperature (25 ± 2 °C), 12 h light/dark cycle and fed on standard feed and water *ad libitum*.

Phytochemical analysis

The extract was subjected to phytochemical analysis for the presence of various constituents. It was screened for the presence of carbohydrates, tannins, glycosides, flavonoids, saponins, anthraquinones, alkaloids according to standard methods (Harborne, 1998).

Acute toxicity studies

Mice of both sexes were used for this test following the Lorke's (1983) model. Four groups of mice were administered intraperitoneally (i.p.) with varying doses of the extract (10, 100, 1000 and 2000 mg/kg, i.p.), while another group which served as control received equivalent volume of normal saline. After treatment, the animals were observed for clinical signs and symptoms of toxicity over a period of 24 hrs. Deaths within this period were recorded, and the LD₅₀ estimated as the square of the lowest lethal dose and the highest non-lethal dose from the second stage of dosing (Vongtau et al., 2004).

Pharmacological evaluation

Studies on castor oil-induced diarrhoea

The mice used in this study were fasted for 18 hrs prior to the experiment but allowed free access to water. The animals were randomly selected and placed in groups of 6 animals per group. Animals in Group 1 were treated with distilled water. Groups 2 – 4 were treated with the extract at doses of 50, 100, and 200 mg/kg, p.o. respectively. While Group 5 was treated with the diphenoxylate 5 mg/kg, p.o. Thirty minutes later, each mouse received 0.5 ml of castor oil. The animals were placed in individual cages lined with white filter paper and observed over a period of 4 hrs. The total number of wet defecations was recorded. The absence of wet faeces was recorded as protection from diarrhoea and the percentage inhibition calculated (Abdulrahman et al., 2004).

Studies on Magnesium Sulphate-induced diarrhoea

Mice were randomly divided into five groups n=6, and fasted of food for 18 hrs prior to the experiment. Group 1 was administered distilled water, Groups 2 – 4 were treated with the extract 50, 100 and 200 mg/kg, p.o respectively, while Group 5 was administered diphenoxylate 5 mg/kg, p.o. After 30 min each mouse received magnesium sulphate 2 g/kg p.o. (Doherty, 1981). The animals were placed in individual cages over white filter paper. The number of wet defecations was recorded for a period of 6 hrs (Mujumdar et al., 2005).

Studies on small intestinal propulsion

Mice were fasted for 18 hrs but allowed free access to water 24 hrs prior to the experiment. The mice were placed into five groups of six animals each. Group 1 received distilled water while groups 2 – 4 were treated with different doses of the extract, 50, 100 and 200 mg/kg and group 5 received atropine 1 mg/kg respectively. After 30 min each mouse was administered orally 0.5 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth). Thirty minutes later, each animal was killed with chloroform and the intestine removed. The intestinal distance moved by the charcoal meal from the pylorus was measured and expressed as a percentage of the distance from the pylorus to the ileocaecal junction for each animal (Gamaniel and Akah, 1996).

Studies on distal colonic propulsion

The mice were fasted for 4 hrs prior to the study. The mice were randomly placed into five groups of 6 animals each. Groups 1 - 3 were treated with the extract at a dose of 50, 100 and 200 mg/kg respectively; while group 4 received distilled water and group 5 received atropine 1 mg/kg. Sixty minutes later, a glass bead of 3 mm in diameter was inserted into the rectum 2 cm from the anus by means of a catheter. The time interval between glass bead insertion and excretion was measured.

Studies on castor oil-induced intestinal fluid accumulation

The mice were fasted for 18 hrs but allowed free access to water. They were randomized and placed in six groups of six animals each. Group 1 and 2 were administered distilled water, Groups 3 – 5 were treated with extract 50, 100, 200 mg/kg p.o. respectively, Group 6 was administered chlorpromazine. Thirty minutes later, animals in groups 2 – 6 received castor oil 0.5ml/ mouse orally. All animals were sacrificed 30 min later; the small intestine was ligated at the pyloric sphincter and the ileocecal junction. The entire small intestine from each mouse was dissected out and weighed. The mean value for each group was calculated. The difference in the weight of intestine in control and castor oil treated groups was considered as the castor oil induced intestinal fluid accumulation (Rao et al., 1997).

Studies on the isolated rabbit jejunum

The adult rabbits were killed by a blow on the head and bled. The abdomen was opened and segments of the jejunum about 2 – 3cm long were removed and dissected free of adhering mesentery. The lumen was flushed with Tyrode solution (Kitchen, 1984) of the following composition (mM): NaCl 8 g, KCl 0.2 g, CaCl₂ 0.2 g, NaHCO₃ 1.0 g, MgCl₂ 0.05 g, NaH₂PO₄ 1.0 g and glucose 1.0g. The tissue was mounted in a 20 ml organ bath containing Tyrode solution at 37 ± 1 °C and aerated with air. A resting tension of 0.5 g was applied. The responses were recorded isometrically on an Ugo Basile Unirecorder 7050 (Italy) through Ugo Basile Isometric Transducer 7004 (Italy) after 1 hr equilibration period during which the physiological solution was changed every 15 min. The effects of graded concentrations of the crude extract were evaluated. The contact time for each concentration of drug was 60 sec, which was followed by washing three times. The tissue was allowed a resting period of 15 min between drug additions.

Statistical Analysis

Results were expressed as mean \pm SEM. Significance was determined using Student's t-test. The difference between means was regarded as significant when $P < 0.05$.

RESULTS

Preliminary phytochemical screening

The fresh extract of *P. muellerianus* gave positive reactions for tannins, carbohydrate, free anthraquinones and flavonoids. LD₅₀ value of extract given i.p. was 547.72 mg/kg.

Effects on castor oil-induced diarrhoea

The extract at 50, 100 and 200 mg/kg and the standard anti-diarrhoeal drug, diphenoxylate 5 mg/kg showed a significant ($P < 0.05$), dose dependent reduction in the number of wet faecal matter produced by the animals (Table 1).

Effects on Magnesium Sulphate-induced diarrhoea

The extract like the standard diphenoxylate significantly ($P < 0.05$) inhibited the frequency of defecation relative to the untreated control mice. There was an inhibition of between 32 – 72% by the extract (Table 2)

Effects on small intestinal transit propulsion

The extract decreased the distance traveled by the charcoal plug in the small intestine as compared to control. This effect is dose dependent (Figure 1)

Effects on distal colonic transit

The methanolic extract of *Phyllanthus muellerianus* caused a delay in distal colonic propulsion which was dose dependent (Figure 2).

Effects on the isolated rabbit jejunum

The extract (0.05 – 3.2 mg/kg) caused a concentration-dependent inhibition of the spontaneous contraction of the rabbit jejunum (Figure 3).

Effects on castor oil induced intestinal fluid accumulation

The extract produced a dose dependent, significant ($P < 0.05$) inhibition of small intestinal fluid accumulation when compared to control (Figure 4).

DISCUSSION

The results showed that the methanolic extract of the leaves of *Phyllanthus muellerianus* exhibited anti-diarrhoeal activity. The extract decreased the frequency of defecation and wetness of the faecal droppings in diarrhoea induced by castor oil when compared to the untreated control group. The effect was similar to that produced by the standard anti-diarrhoeal agent diphenoxylate. The activity of castor oil in inducing diarrhoea is from the action of its active metabolite ricinoleic acid (Mckeon, 1999) formed by hydrolysis of the oil (Iwao and Terada, 1962). Ricinoleic acid produces an irritating action that stimulates the peristaltic activity of the small intestine causing changes in permeability of the intestinal mucosa to electrolytes and water, resulting in a hypersecretory response. These effects are associated with prostaglandin release (Ammon et al., 1974; Capasso et al., 1986; Zavala et al., 1998). Agents that inhibit this activity are known for anti-diarrhoeal effect (Nwafor et al., 2002). The extract protected the mice against MgSO₄ induced diarrhea. MgSO₄ acts by its osmotic properties; it prevents the re-absorption of water and a consequent increase in volume of intestinal content. MgSO₄ promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretions and motility of small intestine, and also, prevents the re-absorption of NaCl and water, indicating that the extract may be affecting the activity of the modulators, prostaglandin and cystokinin (Galvez et al., 1993; Mujumdar et al., 2005). The intestinal propulsive movement in the charcoal meal test was dose dependently inhibited by the extract

Table 1: Effects of the methanolic extract of *Phyllanthus muellerianus* on castor oil-induced diarrhoea.

Treatment	Dose (mg/kg, p.o.)	Number of wet fecal droppings	Inhibition (%)
Water	-	11.00 ± 0.23	-
Extract	50	9.75 ± 0.29	11.36
	100	9.25 ± 0.17	15.91
	200	4.75 ± 0.22*	56.81
Diphenoxylate	5	0.50 ± 0.20*	95.45

Each value represents mean ± S.E.M of 6 mice. * Significantly different from control at P < 0.05.

Table 2: Effects of the methanolic extract of *Phyllanthus muellerianus* on magnesium sulphate-induced diarrhoea.

Treatment	Dose (mg/kg, p.o.)	Number of wet fecal droppings	Inhibition (%)
Water	-	25.00 ± 1.08	-
Extract	50	17.00 ± 1.06*	32
	100	11.00 ± 0.35*	56
	200	7.00 ± 0.79*	72
Diphenoxylate	5	3.00 ± 0.25*	88

Each value represents mean ± S.E.M of 6 mice. * Significantly different from control at P < 0.05.

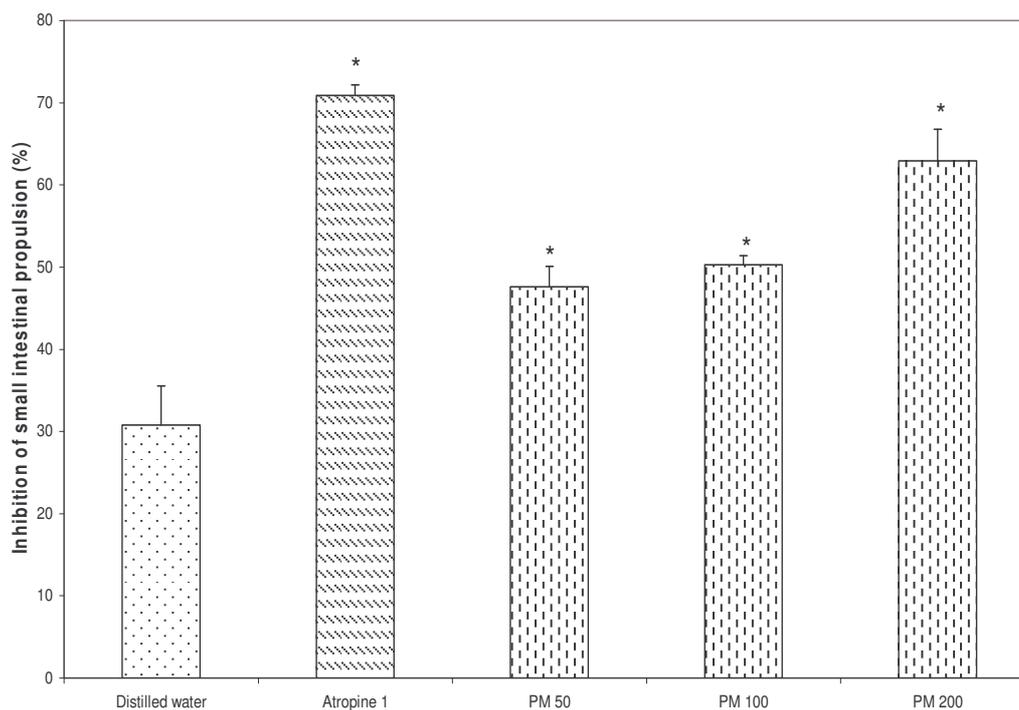


Figure 1: Effects of the methanolic extract of *Phyllanthus muellerianus* (PM) on small intestinal propulsion in mice.

Each column represents mean ± S.E.M of 6 mice. * Significantly different from control at P < 0.05.

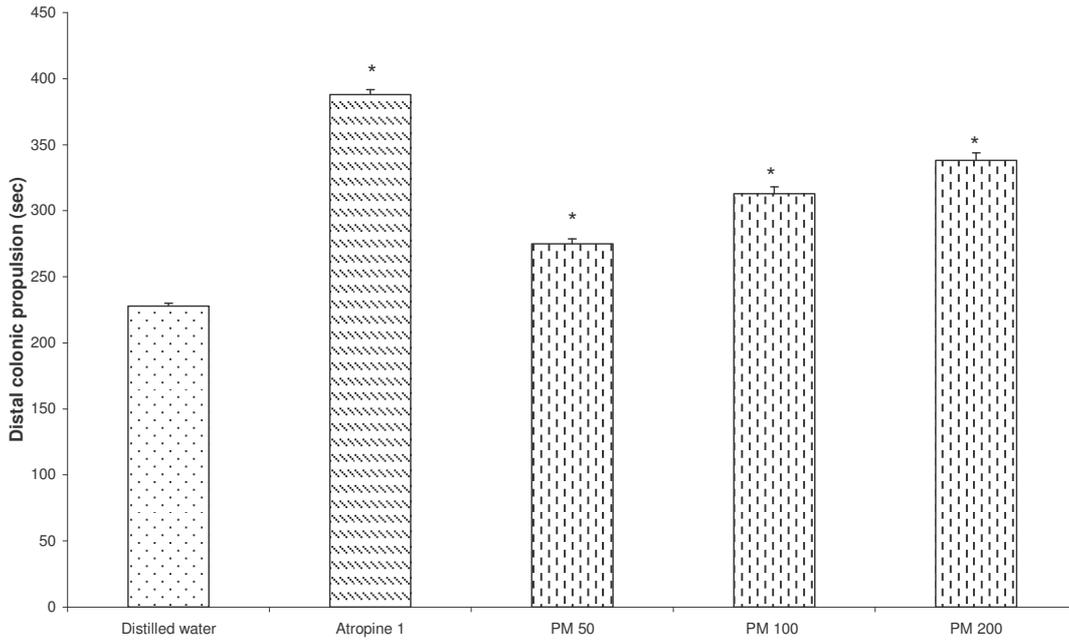


Figure 2: Effects of the methanolic extract of *Phyllanthus muellerianus*(PM) on distal colonic propulsion in mice. Each column represents mean \pm S.E.M of 6 mice. * Significantly different from control at P < 0.05.

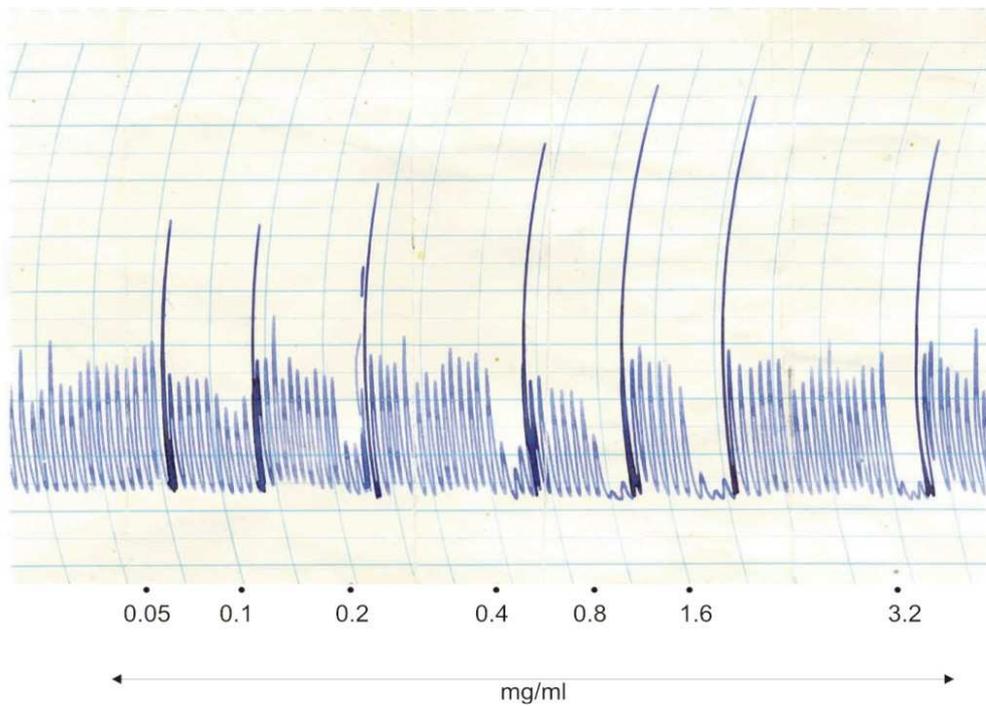


Figure 3: Effects of the methanolic extract of *P. muellerianus* (0.05–3.2 mg/kg) on the spontaneous movement of the rabbit jejunum, n =6.

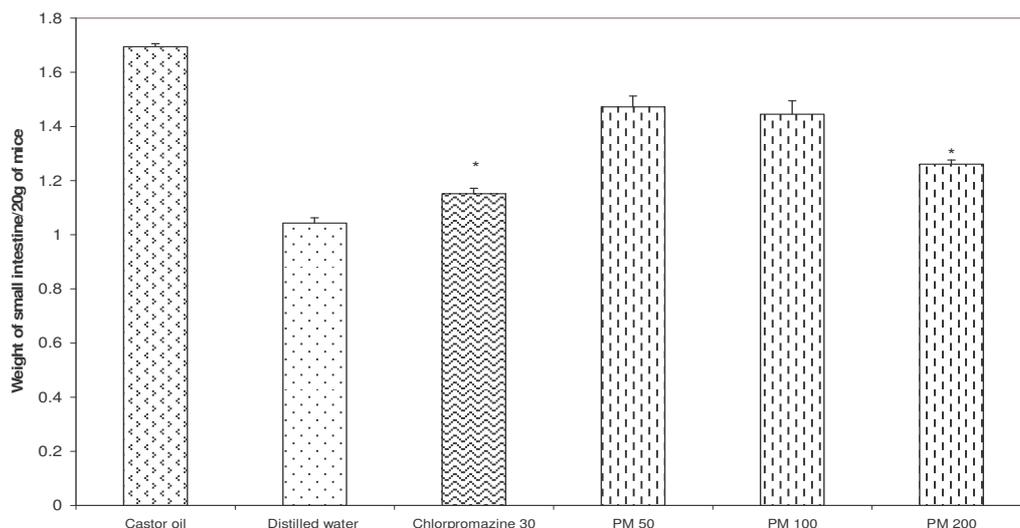


Figure 4: Effects of the methanolic extract of *Phyllanthus muellerianus* (PM) on castor oil-induced intestinal fluid accumulation in mice.

Each column represents mean \pm S.E.M of 6 mice. * Significantly different from castor oil at $P < 0.05$.

in the treated group. The charcoal meal test allows for the comparative evaluation of the degree of inhibition or stimulation of gastrointestinal motility in laboratory animals (Amos et al., 2001). Results also show that the distal colonic transit time was delayed and spontaneous activity of the rabbit jejunum was inhibited in a dose dependent manner. Drugs affecting motility, frequency and consistency of diarrhoea also affect secretion (Di Carlo et al., 1994). The methanolic extract of *Phyllanthus muellerianus* further caused a reduction in castor oil-induced intestinal fluid accumulation in a dose related manner. The enteropooling assay was developed to test the diarrhoea producing property of prostaglandins; inhibition of castor oil induced enteropooling suggests inhibitory effect on prostaglandins (Shook et al., 1989). While there may be non-infectious causes of diarrhoea, it is often a symptom of intestinal infection caused by viruses and bacteria which include *E. coli*. Onocha et al. (2003) have demonstrated the inhibitory effect of *Phyllanthus muellerianus* on some micro-organisms including *E. coli* which is among

the important aetiologic agents of diarrhoea (Katouli et al., 1988). According to the Geiger criteria, for an agent to be classified as an anti-diarrhoeal, it should inhibit the production of wet or unformed stool in animals, inhibit the production of watery stool or fluid evacuation and inhibit gastrointestinal propulsive movement (Offiah and Chikwendu, 1999). The results obtained in this study demonstrate that the methanolic extract of *Phyllanthus muellerianus* has met these criteria, suggesting that *Phyllanthus muellerianus* possess anti-diarrhoeal activity.

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