

Full Length Research Paper

Evaluation of anti-diarrheic properties of the aqueous methanolic extract of *Palisota hirsuta* leaves and its fractions using *in vivo* models

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The anti-diarrheic effects of the methanolic leaf extract of *Palisota hirsuta* (LEP) were evaluated using castor oil-induced diarrhea model and charcoal meal model to evaluate small intestinal transit (SIT). The plant material was extracted using cold maceration with 70% methanol for 48 h and concentrated *in vacuo* and was fractionated using column chromatography. LEP yielded 8.77% w/w material and seven fractions after column chromatography. LEP (100 mg/kg) significantly ($P < 0.05$) decreased total number of fecal output 3 h post-administration of castor oil compared with the 10 ml/kg distilled water treated group (negative control) and caused 46% inhibition of diarrhea compared with diphenoxylate (reference drug). The SIT was significantly ($P < 0.05$) decreased by all doses of LEP used (50, 100, 150 mg/kg), when compared with the negative control and caused 4% inhibition of intestinal fluid accumulation at 50 mg/kg compared with diphenoxylate. LEP fraction 2 (50 mg/kg) caused significant ($P < 0.05$) decrease in SIT compared with the negative control and caused 2% inhibition of intestinal fluid accumulation compared with diphenoxylate. The major phytochemical constituents of LEP fraction 2 were tannins. The methanolic leaf extract of *P. hirsuta* showed promising anti-diarrheic properties, possibly mediated by tannins, thus providing basis for its traditional use as an anti-diarrheic agent.

Key words: *Palisota hirsuta*, anti-diarrhea, small intestinal transit, fluid accumulation.

INTRODUCTION

Diarrhea is a major health problem in developing countries especially sub-Saharan Africa due to a number of different social, political and economic factors which contribute to the constant morbidity from acute and persistent diarrhea, as well as intermittent epidemics of cholera and dysentery (Hamer, 1998). The use of antibiotic in the treatment of certain types of diarrhea especially in people infected with *Escherichia coli* O157:H7 could be counterproductive due to development of haemolytic uremic syndrome (Wong et al., 2000). There

are also concerns for antibiotic resistance (WHO, 2009). It is necessary to identify and evaluate the commonly available natural drugs for efficacy and safety as alternatives to the currently available allopathic anti-diarrheic drugs, which are expensive and can have serious side effects.

Various plant preparations have been used as sources of drugs for treatment of various ailments including diarrhea. The leaves of *Palisota hirsuta* have been used among the Igbo tribe of Eastern Nigeria for the treatment

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of stomach aches, dyspepsia and diarrhea (Oliver, 1960). Roots are used to treat dysentery, anemia and rheumatism while a leaf decoction is used to treat colic (Abbiw, 1990; Mshana et al., 2000). The plant, *P. hirsuta* K. Schum (family Commelinaceae) is variously known as "thunb" in English, "ikpere aturu" or "ikpere anukwu" in various parts of Igbo land, Eastern Nigeria. *P. hirsuta* is a robust herb of forest regions, about 2 to 4 cm high reproducing from seeds (Okezie et al., 1987). Antiinflammatory, antipyretic, analgesic, antimicrobial, anxiolytic, sexual stimulant and antiulcer effects have been reported for this plant (Boakye-Gyasi et al., 2008; Benson et al., 2008; Anaga et al., 2009; Woode et al., 2010; Eke and Anaga, 2013). There has been no scientific report on the anti-diarrheic effects of *P. hirsuta*. We therefore report on the anti-diarrheic effect of *P. hirsuta*

MATERIALS AND METHODS

Animals

Mature white albino mice of both sexes (23 to 34 g) were procured and housed in stainless steel cages. They were fed *ad-libitum* with a standard laboratory animal feed (vital feed®, Grand cereal and oil meals Ltd. Nigeria), except where fasting was necessary. They were maintained in accordance with the recommendation in the guide for the care and use of laboratory animals (DHH, NIH publication No. 85: 23, 1985). All animal experiments were conducted with the permission of the institution's Animal Ethics Committee.

Plant

Fresh green leaves of *P. hirsuta* were collected from Obukpa in Nsukka Local Government Area of Enugu state, South Eastern Nigeria and were confirmed as *P. hirsuta* by a plant taxonomist. The leaves were dried under laboratory conditions at the temperature range of 25 to 27°C for about 10 days and pulverized to a coarse powder (mesh size 1.00 mm) using hammer mill. 382 g of the plant material was extracted by cold maceration using 70% methanol (Sigma-Aldrich Laborchemikalien GMBH, Germany) with intermittent shaking for 48 h. The extract was filtered with Whatmann No.1 filter paper and concentrated *in vacuo* to dryness using a rotary evaporator (Buchi Labortechnik, Switzerland) and was referred to as leaf extract of *P. hirsuta* (LEP). The percentage yield (w/w) of the extract was calculated using the formula:

$$\text{Percentage yield (w/w) of the extract} = \frac{\text{Weight of extracted material}}{\text{Weight of starting material}} \times 100$$

The LEP was separated into fractions using column chromatography (Harbourne, 1991). Briefly, Silica gel 60 G for column chromatography (Vicker, West York England) was used as the stationary phase and 10 g of LEP was adsorbed to it. The column was eluted with petroleum-ether, chloroform, ethyl acetate and methanol in ascending order of polarity as shown in Table 1. Two hundred and thirty five aliquots 10 ml column fractions were collected and spotted on pre-coated silica gel GF254 aluminum plate for thin layer chromatography (TLC) (Merck, Germany) and eluted with chloroform-methanol-ethyl acetate (1:3:1) in a small chromatographic tank to separate the various fractions, based on

their relative mobility on TLC plates and color reactions with UV light (Buchi Labortechnik, Switzerland). This procedure yielded a total of 7 fractions which was used for the experiment. The fractions were concentrated to dryness using a rotary evaporator at 200 millibar at 40°C and were referred to as leaf extract of *P. hirsuta* fractions (LEPfr).

Castor oil induced diarrhea in mice

Twenty five mice were divided into five groups of five mice each. Group 1 received 10 ml/kg distilled water, group 2 received 5 mg/kg diphenoxylate and groups 3 to 5 received 50, 100 and 150 mg/kg, respectively of LEP. Thirty minutes post treatment 0.5 ml of castor oil (Bell sons and co. Southport England) was administered to all the animals. The animals were kept in individual cages and floor of which were lined with blotting paper, and the number of both normal and watery droppings were counted for each mouse every hour over a period of 4 h. Mean of the stools passed by the treated groups were compared with that of the control (Vander et al., 2007).

Small intestinal transit (SIT) and fluid accumulation (FA)

Twenty five mice were divided into five groups of five and were fasted for 16 h before the experiment. Group 1 received 10 ml/kg distilled water, group 2 received 5 mg/kg diphenoxylate, while groups 3 to 5 received 50, 100 and 150 mg/kg LEP. In addition, for the LEPfr, 27 mice of both sexes were grouped into 9 groups of 3 mice each while the controls received either 10 ml/kg distilled water or 5 mg/kg diphenoxylate, groups 3 to 9 received 50 mg/kg of LEPfr 1 to 7, respectively. One hour post treatment, a standard charcoal meal (0.5 ml of 5% activated charcoal suspension in 5% gum acacia) was administered to all the animals. The animals were sacrificed 30 min post-administration of charcoal meal under mild ether anesthesia and the intestine immediately isolated and ligated at the pyloric sphincter and at the ileo-cecal junction. The SIT (the peristaltic index) of each mouse was expressed as percentage of distance travelled by the charcoal meal relative to the total length of the small intestine from the pyloric sphincter to the ileo-cecal junction of each mouse. The FA was determined by weighing the intestine and its contents, then milking out the intestinal contents, and finally reweighing the empty intestine to determine the final weight. The difference between full and empty intestine was determined. Percentage inhibition of FA was calculated (Rao et al., 1997). The mean fecal output and the mean SIT were analyzed using one way analysis of variance and variant means were separated post-hoc using the least significant difference (LSD). Results were expressed as means ± standard error of means (SEM) and significance was accepted at the probability level ($p < 0.05$). The ratio of normal to watery droppings, percentage inhibition of diarrhea and percentage inhibition of intestinal fluid accumulation were expressed as percentages.

Spot phytochemical analysis of LEPfr

Phytochemical analysis of LEPfr was carried out using standard procedure (Trease and Evans, 1999). LEPfr was tested for the presence of alkaloids, flavonoids, tannins, glycosides, starch and carbohydrates.

RESULTS

The methanolic leaf extract of *P. hirsuta* yielded 8.77% w/w material which was dark green in color, pasty in

Table 1. Solvent system for chromatographic separation of LEP.

Pet ether %	Chloroform %	Ethyl acetate %	Methanol %
100	-	-	-
70	30	-	-
50	50	-	-
20	70	10	-
-	80	20	-
-	60	40	-
-	40	50	10
-	20	60	20
-	-	70	30
-	-	50	50
-	-	30	70
-	-	10	90
-	-	-	100

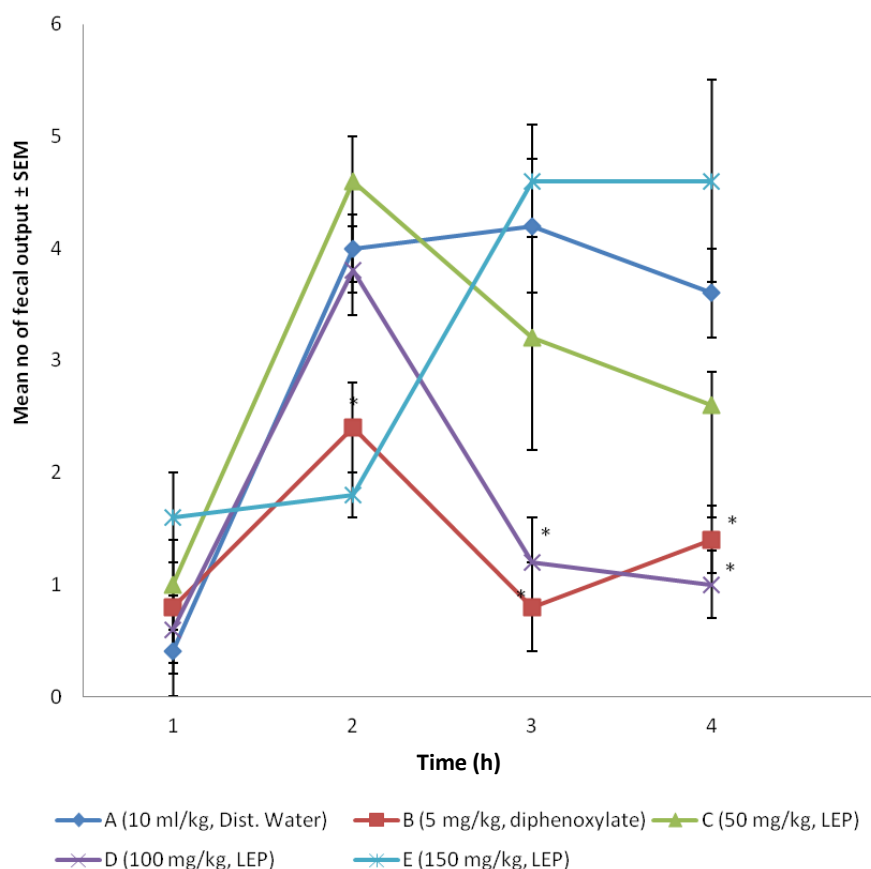


Figure 1. Effect of LEP on castor oil-induced diarrhea in mice. Mean ± SEM* is significant at $p < 0.05$ compared with 10 ml/kg distilled water.

consistency and had a sharp pungent smell. Chromatographic separation of LEP yielded seven fractions. Spot phytochemical analysis of LEPfr2, which was the most active fraction showed the presence of tannins. The result of the castor oil induced diarrhea in mice as

shown in Figure 1 showed that there was no significant difference in the mean number of fecal output among the groups one hour post-administration of castor oil. Fecal output at this stage was only the solid type. At two hours post-administration of castor oil, there was a general

Table 2. Effect of LEP on fecal consistency in mice.

Group	% solid droppings	% Watery droppings	% Inhibition of diarrhea
A	18	82	0
B	41	59	56
C	42	58	7
D	61	39	46
E	19	81	0

A: 10 ml/kg distilled water, B: 5 mg/kg diphenoxylate, C – E (50, 100 and 150 mg/kg) LEP, respectively.

Table 3. Effect of LEP on small intestinal transit in mice.

Group	% Small intestinal transit (SIT)
10 ml/kg b.w. dist. water	80.29±7.88
5 mg/kg b.w. diphenoxylate	55.10±9.53*
50 mg/kg b.w. LEP	45.71±6.02*
100 mg/kg b.w. LEP	49.50±2.45*
150 mg/kg b.w. LEP	45.84±5.31*

Mean ± SEM* is significant at $p < 0.05$ compared with 10 ml/kg distilled water.

Table 4. Effect of LEP on intestinal fluid accumulation in mice.

Group	% inhibition of intestinal fluid accumulation
A (10 ml/kg, distilled water)	0
B (5 mg/kg, diphenoxylate)	0
C (50 mg/kg, MLEPH)	4
D (100 mg/kg, MLEPH)	0
E (150 mg/kg, MLEPH)	0

increase in the mean number of fecal output among the groups, but diphenoxylate (5 mg/kg b.w.) significantly ($p < 0.05$) decreased the number of fecal outputs when compared with the LEP treated groups. There was also significant ($p < 0.05$) decrease in the number of fecal output in the LEP 100 (mg/kg b.w.) and diphenoxylate (5 mg/kg b.w.) treated groups 3 and 4 h post-administration of castor oil. Comparison of the ratio of solid to watery stool (Table 2) showed that LEP 100 mg/kg b.w. produced 39% watery stools against 59% by diphenoxylate (5 mg/kg b.w.) while comparison of the percentage inhibition of diarrhea among the groups showed that diphenoxylate (5 mg/kg b.w.) caused 56% inhibition as against 46 and 7% by 100 and 50 mg/kg LEP, respectively.

LEP at doses 50, 100 and 150 mg/kg b.w. and 5 mg/kg b.w. diphenoxylate significantly ($p < 0.05$) decreased the SIT when compared with the distilled water control group (Table 3). The effect of LEP on FA is shown in Table 4. LEP (50 mg/kg b.w.) caused 4% inhibition of FA compared with diphenoxylate. Table 5 presents the effects of LEPfr on the SIT and it showed that LEPfr2 (50

mg/kg b.w.) and diphenoxylate (5 mg/kg b.w.) significantly ($p < 0.05$) decreased the SIT when compared with the distilled water group. The effect of LEPfr on FA (Table 6) showed that LEPfr2 and LEPfr3 caused 2 and 7% inhibition of intestinal fluid accumulation when compared with diphenoxylate.

DISCUSSION

Diarrhea is as a result of imbalance between absorptive and secretory mechanisms in the intestinal tract involving two components; motility and secretory components (Chitme et al., 2004). A good anti diarrheic agent must have significant effect on any or both of these components. Castor oil causes irritation of the intestinal mucosa via liberation of ricinoleic acid and the irritation leads to release of prostaglandin which causes excessive intestinal fluid secretion and motility. Normal intestinal fluid absorption is also impaired by castor oil through inhibition of intestinal Na^+K^+ ATPase activity (Gaginella et

Table 5. Effect of LEPfr on small intestinal transit in mice.

Group	% Small intestinal transit (SIT)
10 ml/kg b.w. dist water	76.28±3.3
5 mg/kg b.w. diphenoxylate	54.44±3.8*
50 mg/kg b.w. LEPfr1	76.24±9.3
50 mg/kg b.w. LEPfr2	54.37±4.2*
50 mg/kg b.w. LEPfr3	60.29±3.5
50 mg/kg b.w. LEPfr4	68.81±10.7
50 mg/kg b.w. LEPfr5	82.58±4.2
50 mg/kg b.w. LEPfr6	68.50±4.3
50 mg/kg b.w. LEPfr7	58.42±7.5

Mean ± SEM* is significant at $p < 0.05$ compared with 10 ml/kg distilled water.

Table 6. Effect of LEPfr on intestinal fluid accumulation (FA) in mice.

Group	% Inhibition of FA
A (10 ml/kg, distilled water)	0
B (5 mg/kg, diphenoxylate)	0
C (50 mg/kg, LEPfr1)	0
D (50 mg/kg, LEPfr2)	2
E (50 mg/kg, LEPfr3)	7
F (50 mg/kg, LEPfr4)	0
G (50 mg/kg, LEPfr5)	0
H (50 mg/kg, LEPfr6)	0
I (50 mg/kg, LEPfr7)	0

al., 1978).

Anti-diarrheic effect of LEP became apparent 3 h post administration of castor oil and sustained till the end of the experiment. Pretreatment of mice with LEP possibly resulted in amelioration of irritation and inflammation of intestinal mucosa induced by ricinoleic acid liberated from castor oil, thus leading to decrease in intestinal motility and secretion for an overall reduction in the rate of passage of watery stool. It is also possible that LEP activated the intestinal Na^+K^+ -ATPase activity to enhance normal fluid absorption, thereby reducing diarrhea.

The motility and secretory activity of the gastro intestinal tract (GIT) are controlled by various neurotransmitters secreted by the enteric nervous system. Some are excitatory like acetylcholine and will lead to increased gut motility and secretion while some are inhibitory like noradrenaline and will lead to decreased gut motility and secretion (Guyton and Hall, 2001). LEP at all doses and its fraction 2 (LEPfr2, 50

mg/kg b.w) which was the most active fraction significantly slowed down charcoal meal transit in the gastrointestinal tract, showing that LEP could have inhibitory effects on the excitatory neurotransmitters in the gastrointestinal tract thus leading to relaxation of the gut muscles and slowing down motility. There was also a

4% decrease in intestinal fluid accumulation at 50 mg/kg b.w LEP and 2% by LEPfr2, pointing to mild antisecretory effect. The effects of LEP on the small intestinal transit and fluid accumulation tend to decrease with purification, signifying that other phytochemical constituents of LEP are important for effective anti-motility and antisecretory effects. It also seems that beyond 100 mg/kg, effectiveness of LEP as a gastrointestinal protectant decreases.

It has been found that anti-diarrheic properties of medicinal plants are due to tannins, flavonoids alkaloids, saponins, sterols, triterpenes and reducing sugars (Longanga-Otshudi et al., 2000; Teke et al., 2010). The major phytochemical constituents of LEP were found to be tannins, flavonoids glycosides and proteins (Anaga et al., 2009). The flavonoids, tannins or glycosides may have mediated the anti-diarrheic effects of LEP. Phytochemical analysis of LEPfr2 (the most active fraction) showed that the major constituents were tannins, therefore suggesting that the anti-diarrheic effects of *P. hirsuta* leaves were mediated mainly by the tannin content. Tannin containing drugs will precipitate proteins and have been used internally for the protection of inflamed surfaces of mucous membranes (Trease and Evans, 1999). The astringent actions of tannins function to precipitate microproteins on inflamed mucous membranes, thereby forming a protective layer over the mucosal lining and protect the underlying mucosa from irritants and toxins (Clinton, 2009).

Conclusion

Our results suggest that *P. hirsuta* possesses promising anti-diarrheic effects mediated mainly by the tannin component. The effects on the small intestinal transit and fluid accumulation suggest that the anti-diarrheic mechanism was through myo-relaxant effect than anti secretory. These effects of *P. hirsuta* seem to provide basis for its traditional use as an anti-diarrheic agent. We recommend further pharmacological and toxicological investigation into the anti-diarrheic properties of *P. hirsuta*

leaves in order to exploit the full potential of this medicinal plant as an anti-diarrheic agent.

ABBREVIATIONS

LEP, Leaf extract of *Palisota hirsuta*; **LEPfr**, leaf extract of *P. hirsuta* fraction; **SIT**, small *hirsuta* intestinal transit; **FA**, fluid accumulation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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