

Original Research Article

Antidiarrheal activity of methanol leaf extract of *Lophira lanceolata* Tiegh (Ochnaceae)

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Abstract

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This study investigated the effect of methanol leaf extract of *Lophira lanceolata* for antidiarrheal and spasmolytic properties in experimental albino Wistar rats. The methanol extract (ME) obtained by 72 h maceration was evaluated for antidiarrheal activity using Castor oil induced diarrhea, GIT transit of Charcoal meal model in rodents and isolated guinea pig ileum model. The extract exhibited antidiarrheal and antispasmodic activity in a non-dose dependent manner. At 100 mg/kg, extract produced highest percentage diarrhea protection (63.33%) when compared to the negative control while 79.95% protection was recorded with loperamide. The extract exhibited highest anti-motility effect at 200 mg/kg of the extract (giving 5.14% motility inhibition as against 31.34% of atropine). The extract at 500µg completely blocked contraction induced by 5µg of acetylcholine. Similarly, 1µg of Atropine also blocked contraction induced by 5µg of acetylcholine. The results showed that the methanol leaf extract of *Lophira lanceolata* has antidiarrheal and antispasmodic activity which supports its folkloric medicinal use.

Keywords: Acetylcholine, Antidiarrheal, Antispasmodics, Guinea pig, *Lophira lanceolata*.

INTRODUCTION

Diarrhea is an increase in the frequency of bowel movements or a decrease in the form of stool or change in the consistency of stool (greater looseness of stool). It can also be defined as an increase in the volume of stool or frequency of defecation. World Health Organization (WHO) defined diarrhea as three or more watery or loose bowel movements in a 24 hour period (WHO, 2014). Diarrhea is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside of the digestive system (Christian, 2014).

Worldwide, diarrhea claims several million lives annually, mostly those of infants. In 2004 approximately

2.5 billion cases of diarrhea occurred worldwide which results in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia. Diarrhea remains the second leading cause of infant mortality (16%) after pneumonia (17%) in this age group (Teke et al.; 2003).

Lophira lanceolata among other folkloric medicinal uses has been used to treat diarrhea, dysentery, menstrual pain. However, these claims are yet to be studied.

The goal of this study was to investigate the *Lophira lanceolata* for antidiarrheal activities.

MATERIALS AND METHODS

Animals

Male Guinea pig (350g) and Wistar albino rats (115-210g) of either sex were obtained and housed in cages, acclimatized under normal laboratory environmental conditions and feed in the animal house facility of the Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were maintained freely on standard pellets and water. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Plant Collection, Authentication and Preparation

The fresh leaves of *Lophira lanceolata* was collected from Enugu state, Nigeria in August, 2013. The plant was identified and authenticated by Mr. A Ozioko, a Taxonomist and staff of the International Centre for Ethno medicine and Drug Development (InterCEDD) Nsukka, Nigeria. The fresh leaves were air-dried at room temperature properly and then grounded into powder using a grinder and weighed using a weighing balance.

Extraction Procedure

About 5kg of the powdered material was macerated with 20 L of 80% methanol for 72 h with constant shaking; the resultant mixture was filtered using a nylon cloth and the filtrate concentrated to dryness at room temperature. The extract was stored in a freezer at -15°C, required concentrations were reconstituted as needed.

Determination of Percentage extractive Yield

Percentage yield was calculated as the weight of the filtrate divided by the total weight of the ground powder in percentage.

Yield (%) = [wt of extract (g)/wt of plant material (g)] x 100.

Chemicals, solvents and reagents

Methanol (Sigma Aldrich, Germany), Tween 80, distilled

water, Charcoal, tragacanth, NaCl, KCl, MgCl₂, NaHCO₃, NaH₂PO₄, Glucose, CaCl₂. Loperamide (Drugfield Nigeria), Castor oil, atropine sulphate, Acetylcholine, Histamine, and Promethazine.

Equipment

Analytical weighing balance (Furi; FEJ-600, China), kymograph (Ugo Basile, Germany), tissue organ bath (Ugo Basile, Germany).

Pharmacological Tests

Castor oil-induced diarrhea in rats

Thirty Swiss albino rats of either sex were randomly selected into 5 groups (n=6) and fasted for 18 hours (Sunil et al.; 2001).

Group I (negative group) received 2ml/kg of distilled water, Groups II,III,IV received 100,200, and 400mg/kg of plant extract respectively while group V (positive group) received Loperamide (4m/kg) per orally. After 1 hour of drug treatment, 2 ml of Castor oil was given orally to each rat. The onset time was noted and diarrhea defecations were observed hourly for 6hours. Inhibition of defecation (%) was calculated.

$$\text{Inhibition of defecation (\%)} = \frac{M_c - M_d}{M_c} \times 100$$

Where M_c: mean defecation caused by castor oil, M_d: mean defecation caused by drug or extract (Williamson, 1996)).

Gastrointestinal Transit Time in rats

Experimental rats of either sex (115-150g) grouped randomly into 5 groups of (n=6) and fasted for 18hours (Sunil et al.; 2001).

Group I (negative group) received 2ml/kg of distilled water, Groups II, III, IV received 100, 200, and 400mg/kg of plant extract orally while group V (positive group) was given 4mg/kg of atropine orally. After 1hour of drug administration, each animal was given 2ml of standard charcoal meal (10% activated charcoal suspension in 10% tragacanth gum).

The rats were sacrificed 1hour after the administration of the charcoal meal, the abdomen were opened and the small intestine was immediately isolated. The length of the intestine from pylorus to the caecum (LSI) and the distance travelled by charcoal (LM) were measured.

The peristaltic index (PI) and % inhibition for each rat were calculated using relations below;

$$\% \text{ Inhibition} = \frac{\text{Control-Test}}{\text{Control}} * 100; \text{ PI} = \frac{\text{LM}}{\text{LSI}} * 100;$$

PI=Peristaltic Index; LM=Length Charcoal Meal; LSI=Length of Small Intestine.

In vitro testing on Guinea pig Ileum

The isolated tissue preparations according to the technique of Perry (Perry, 1982) and Williamson *et al.* (de la Puerta and Herrera 1995) were employed.

A male guinea pig (350g) fasted for 18h, was sacrificed to obtain a 2cm long pieces of ileum for the experiment. A piece of guinea pig ileum was tied with the aid of thread at both ends and suspended in a thermoregulated 50 ml organ bath, maintained at 37°C, containing a Tyrode solution. (Galvez, 1996; Onyeto *et al.*, 2014).

The suspended ileum was allowed to equilibrate for 45 minutes and the solution was refreshed every 15 minutes with Tyrode solution. After the initial equilibration period, acetylcholine (5µg) then followed by histamine (5µg) as controls. The methanol extract of *Lophira lanceolata* at organ bath concentrations of 1µg/ml, 10µg/ml, 1000µg/ml, 2000µg/ml and 4000µg/ml were added alone for effects, then in co-administration with acetylcholine (5µg) and histamine (5µg) respectively until complete blockade was observed in each case. The dose of the plant extract that completely blocked acetylcholine (5µg) and histamine (5µg) were noted. Then varied doses of promethazine and atropine were then added with corresponding administration of histamine (5µg) and acetylcholine (5µg) respectively and the point of complete blockades were noted respectively.

Isometric contractions were recorded on kymograph paper. Relaxation (%) was calculated using the relation;

$$\text{Relaxation (\%)} = \frac{H_o - H_r}{H_o} * 100$$

Where H_o=Original response height, H_r=height of relaxation response.

The antihistamine effects of the ME extract at different doses were compared with promethazine (1µg and 5µg) and the anticholinergic effects of the ME extract at different doses were equally compared with atropine (1µg and 5µg).

STATISTICAL ANALYSIS

Results obtained were subjected to one-way analysis of variance (ANOVA) using Graph pad Prism Version 5, followed by Dunnett's post hoc test and P<0.05 was considered significant. The results are expressed as

Mean ± SEM.

RESULTS

Percentage Extractive Yield

The extraction process afforded 331 g (6.55% w/w) of the methanol extract.

Phytochemical Constituents of Extract

The methanol leaf extract of *Lophira lanceolata* gave positive test for flavonoid, alkaloid, glycoside, saponins, and terpenoids, reducing sugar, oils and carbohydrates (Tunaru, 2012).

Pharmacological Tests

Acute Toxicity Test

Oral administration of methanol extract of *L.lanceolata* up to 5 g/kg caused no death in mice. Therefore, the oral LD₅₀ of the aqueous extract in mice was >5 k/kg. Also there were no signs of obvious behavioral and physical adverse effects (Tunaru, 2012).

Effect of Extract on Castor oil-induced diarrhea in rats

The extract inhibited castor oil-induced diarrhea in non-dose dependent manner with highest inhibition exhibited at 100mg/kg. The degree of inhibition was expressed as percentage inhibition of defecation (Table 1).

Effect of Extract on GIT Transit Time of Charcoal meal

The extract caused a non-dose dependent decrease in the movement of charcoal meal in rats. The degree of inhibition of the peristaltic movement expressed as percentage inhibition of movement was highest at 200mg/kg dose of extract (Table 2).

Effect of Extract on guinea pig ileum

Atropine and promethazine at 1µg and 5µg respectively inhibited 5µg of acetylcholine and 5µg histamine

Table 1. Effect of Extract on Castor oil-induced diarrhea in rats

Groups	Treatment	Dose mg/kg	Mean defecation in 6 hours \pm SEM	%Inhibition of defecation
I	Control	2ml	0.8333 \pm 0.3752	0.00
II	ME	100	0.1667 \pm 0.1361	79.95
III		200	0.3056 \pm 0.2412	63.33
IV		400	0.4722 \pm 0.2667	43.34
V	Loperamide	4	0.1111 \pm 0.1111	86.67

ME, Methanol extract. The values are mean \pm SEM., n=6, *P<0.05 vs control.

Table 2. Effect of Extract on GIT Transit Time of Charcoal meal

Groups	Treatment	Dose mg/kg	Length of Intestine \pm SEM(cm)	Movement Of Charcoal \pm SEM(cm)	%Inhibition of movement
I	Control	2ml	98.0000 \pm 2.6330	98.000 \pm 2.633	0.00
II	ME	100	105.0000 \pm 2.1910	100.800 \pm 2.713	4.00
III		200	97.3300 \pm 3.0070	92.330 \pm 5.220	5.14
IV		400	103.8000 \pm 3.7600	102.400 \pm 4.007	1.35
V	Atropine	3	101.7000 \pm 0.6667	69.830 \pm 15.110*	31.34

ME, Methanol extract. The values are mean \pm SEM., n=6, *P<0.05 vs control.

Table 3. Result of Responses of extract on acetylcholine induced contractions

Dose	Height of Response(cm)	% Relaxation	Response
5 μ g Ach	4.20	-	Contraction
5 μ g Ach	4.00	-	Contraction
Mean	4.10	-	
5 μ g Ach+10 μ g ME	0.40	90.00	Relaxation
5 μ g Ach+50 μ g MEL	0.30	92.00	Relaxation
5 μ g Ach+100 μ g MEL	0.70	82.93	Relaxation
5 μ g Ach+500 μ g MEL	0.00	100.00	Blocked
5 μ g Ach+1mg MEL	0.00	100.00	Blocked
5 μ g Ach+1 μ g Atropine	0.00	100.00	Blocked
5 μ g Ach+5 μ g Atropine	0.00	100.00	Blocked

ME, Methanol extract. The values are mean \pm SEM., n=6, *P<0.05 vs control.

Table 4. Result of Responses of extract on Ach induced contractions

Dose	Height of Response(cm)	% Relaxation	Response
5 μ g Histamine	3.30	-	Contraction
5 μ g Histamine	3.50	-	Contraction
Mean	3.40	-	
5 μ g Hist+10 μ g ME	3.70	0.00	Contraction
5 μ g Hist+50 μ g ME	3.30	2.94	Relaxation
5 μ g Hist+100 μ g ME	3.20	5.88	Relaxation
5 μ g Hist+500 μ g ME	3.00	11.76	Relaxation
5 μ g Hist+1mg ME	2.60	23.53	Relaxation
5 μ g Hist+5mg ME	2.50	26.47	Relaxation
5 μ g Hist+10mg ME	1.70	50.00	Relaxation
5 μ g Hist+50mg ME	0.50	85.29	Relaxation
5 μ g Hist+100mg ME	0.00	100.00	Blocked
5 μ g Hist+1 μ g Promethazine	0.30	91.18	Relaxation
5 μ g Hist+5 μ g Promethazine	0.00	100.00	Blocked

ME, Methanol extract. The values are mean \pm SEM., n=6, *P<0.05 vs control.

contractions respectively while 500µg and 100mg of extract caused complete blockade of 5µg of Acetylcholine and 5µg histamine contractions respectively (Tables 3 and 4). At 1µg and 5µg doses of atropine and promethazine respectively, there were inhibitions of concentrations produced by acetylcholine and histamine each at 5µg whereas there was a complete blockade of the agonistic effects of both drugs at same dose as above by 500µg and 100µg of the extract respectively.

DISCUSSION

Phytochemical studies of methanol extract of leaves of *Lophira lanceolata* revealed the presence of abundance of flavonoids. Alkaloids, glycosides and oil were moderately present while saponins, terpenes, reducing sugars, carbohydrate and acidic compounds occurred in relatively small quantities (Tunaru, 2012).

Acute toxicity test suggests a considerable good safety profile such that even 5000mg/kg/day oral dose, of the leaf extract is still safe for experimental rats as reported by Onyeto *et al* (Tunaru, 2012).

Eighty percent of castor oil is ricinoleic acid, a fatty acid that binds to receptors located on the smooth muscle cells of the intestines, once locked onto those receptors, ricinoleic acid causes powerful contractions, explaining castor oil's reputation as a stimulant laxative (Rouf, 2003). Castor oil is also reported to induce diarrhea by increasing the volume of intestinal content by prevention of the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in the stimulation of secretion, thereby, preventing the reabsorption of sodium chloride and water. Castor oil therefore incorporates both secretory and motility diarrhea (Janssen, 2014). Inhibitors of prostaglandin synthesis are known to delay diarrhea induced with castor oil (Williamson, 1996)).

Loperamide is an opioid-receptor agonist that acts on the µ-opioid receptors in the mesenteric plexus of the large intestine by decreasing the activity of the mesenteric plexus, which in turn decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall. This increases the amount of time substances stay in the intestine.

The result of castor oil induced diarrhea studies showed that the methanol leaf extract has comparable inhibitory potentials on diarrhea with the antidiarrheal drug, loperamide. The observed antidiarrheal effect of extract may be linked to the same mechanism as of loperamide action or possibly inhibition of prostaglandin biosynthesis.

In the motility studies using charcoal meal, the extract

did not show significant percentage inhibition. This may suggest that its antidiarrheal effects of the extract may be due to other mechanisms outside the anti-motility. However, this contrasts its behavior in test for spasmolytic activity using guinea pig ileum where it showed significant inhibition of smooth muscle contractions produced by acetylcholine antagonist like atropine.

The spasmolytic activity of methanol extract of leaves of *Lophira lanceolata* was tested using smooth muscle preparations. Acetylcholine is a neurotransmitter released by the parasympathetic nervous system. It mediates its action in the gut by stimulation of M₃ receptors, hence, regulating the peristaltic movement of the gut. Muscarinic antagonists like atropine inhibits action of acetylcholine thus leading to decrease in gut peristalsis.

Although the result of the studies showed that the extract has 500 times and 20,000 times less activity than atropine and promethazine respectively, it antagonized the activity of acetylcholine more. This further suggests the possibility of inhibition of smooth muscle via muscarinic antagonistic activity like atropine.

CONCLUSION

The leaf extract of *Lophira lanceolata* possess antidiarrheal and some spasmolytic effects. These findings provide a scientific proof for the use of this plant in the treatment of diarrhea in humans and livestock. Further studies are required to isolate and characterize the exact active constituents of the leaf extract responsible for its anti-diarrhea activity.

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