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Antidiarrheal Activity of *Lannea coromandelica* Linn. Bark Extract

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Abstract: The present study was designed to investigate the antidiarrheal potential of the methanolic extract of *Lannea coromandelica* bark (MLCB). The extract studied for antidiarrheal property using castor oil and magnesium sulphate induced diarrheal model and charcoal induced gastrointestinal motility as well as PGE_2-induced enterolooping test in mice. In addition, activities against some causative diarrheal pathogenic bacteria were also determined. At the doses of 100 and 200 mg/kg body weight, MLCB extract significantly reduced the frequency and severity of diarrhea in test animals throughout the study period in a dose dependent manner and also showed a significant ($p<0.05$) reduction in the gastrointestinal motility in charcoal meal test as well as PGE_2-induced intrafluid accumulation. MLCB extract also displayed strong antibacterial effect against some diarrhoic pathogenic bacteria and highest activity was found against *Escherichia coli* (zone of inhibition 15.59 ± 0.22 mm). Altogether, these results suggest that the *Lannea coromandelica* bark extracts could be used as a potential antidiarrheal agent.

Key words: Diarrhea · Flavonoid · Antibacterial · *Lannea coromandelica*

INTRODUCTION

Diarrhea is an alteration in the normal bowel movement, characterized by increased frequency of bowel sound and movement, wet stool and abdominal pain [1]. Diarrhea, may be acute or chronic. With acute diarrhea being the most common is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions can contribute a lot. Now a days, rotavirus is the major causative agent for infectious diarrhea, particularly in young children, however, other viral (adenovirus, enterovirus and norovirus), bacterial (*Escherichia coli, Salmonella sp. Shigella sp. Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens [2]. Oral rehydration therapy (ORT) has been identified as a key factor in the decline of child mortality rate due to diarrhea, although it does not reduce the volume or duration of diarrhea [3]. Likely, antibiotics and gut motility suppressing agents bid the other treatment option, wherein reverse dehydration, shorten the length of illness and reduce the period of time when an individual is infected [4]. Treatment with pharmacological agents that are pathogen specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhea [5].

The genus Lannea belongs to the family Anacardiaceae and consists of 40 species. *Lannea coromandelica* L. is a deciduous tropical tree widely distributed in Bangladesh, India and some other tropical countries. The bark of *L. coromandelica* was used for skin disease [6], injuries and hematochizia [7], hypotensive [8], antifungal and antibacterial [9] agent. The stem bark of this plant was used by the Garo tribes in Madhapur porest region of Bangladesh to treat seminal weakness and excessive seminal emission [10]. The leaf juice was orally taken to relieve ulcers and pain [11]. It was also claimed to used as antidote in coma caused by narcotics, to treat dyspepsia, gout, dysentery, sore eyes, leprosy and bruises [12]. Literature reviews indicated that no studies in antidiarrheal effects of the bark of *L. coromandelica* have so far been undertaken. Taking this

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in view and as a part of our ongoing research [13] on Bangladeshi medicinal plants, the present study aimed to evaluate the antidiarrheal activity of the methanolic bark extracts of *Lannea coromandelica*.

MATERIALS AND METHODS

Plant Material: The bark of the plant of *Lannea coromandelica* Linn was collected from the botanical garden of Pharmacy department, Jahangirnagar University, Bangladesh during January 2010. The plant material was taxonomically identified by the National Herbarium of Bangladesh whose voucher specimen no. JU290 is maintained in our laboratory for future reference.

Preparation of Plant Extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powder material (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (75 g).

Chemicals: Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). Galic acid and quercetin, were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

The Amount of Phenolic Compounds and Flavonoids: The total phenolic content of extract was determined using Folin-Ciocalteu reagent [14]. Extracts (100 µl) were mixed with the Folin-Ciocalteu reagent (500 µl) and 20% sodium carbonate (1.5 ml). The mixture was shaken thoroughly and made up to 10 ml with distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

The flavonoids content was determined by aluminium chloride colorimetric method [15]. The different concentration of extracts (0.5 ml) were separately mixed with 95% ethanol (1.5 ml), 10% aluminum chloride (0.1 ml), 1M potassium acetate (0.1 ml) and distilled water (2.8 ml). After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. All the determinations were carried out in duplicates. These data were used to estimate the flavonoid contents using a standard curve obtained from various concentration of quercetin.

Acute Toxicity Study: Animals were divided into groups of five mice each. The test was performed using increasing doses of both test extracts, given orally, in a 10 ml/kg volume to different groups serving as test groups [16]. Another group of mice was administered saline (10 mL/kg, p.o.) as negative control. The mice were allowed food *ad libitum* during the 24 h test and kept under regular observation for mortality.

***In vivo* Anti-Diarrheal Activity**

Castor Oil-induced Diarrhea: The experiment was performed according to the method described by Shoba and Thomas [17]. Briefly, mice fasted for 24 h were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% CMC (10 ml/kg, *p.o.*), groups III-IV received orally MLCB extract (100 and 200 mg/kg), respectively. Group II was given Loperamide (3 mg/ kg, *p.o.*) in suspension. After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 h and the characteristic diarrhoeal droppings were recorded.

Magnesium Sulphate-Induced Diarrhea: Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, *p.o.*) to the control group, loperamide (3 mg/kg) to the positive control group and the methanol extract (MLCB) at the doses of 100 and 200 mg/kg to the test groups [18].

Effect on Gastrointestinal Motility: Animals were divided into four groups of five mice each and each animal was given orally 1 ml of charcoal meal (5% activated charcoal suspended in 1% CMC) 60 min after an oral dose of drugs or vehicle. Group I was administered 1% CMC (10 ml/kg) and animals in groups III-IV received extract of MLCB at the dose of 100 mg/kg and 200 mg/kg body weight, respectively. Group II received atropine sulfate (0.1 mg/kg,) as the standard drug. After 30 min, animals were killed by light ether anaesthesia and the intestine was removed without stretching and placed lengthwise on

moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine [19].

PGE_2-Induced Enteropooling: The method of Robert *et al.* [20] was applied. Overnight fasted mice were divided into five groups of 5 animals each. Group I was given 2% gum acacia and kept as a control. Groups III-IV received 100 and 200 mg/kg p.o. of MLCB extracts, respectively. Group II served as a vehicle control and received 2% gum acacia plus PGE_2 (0.5 ml of 100µg/kg, i.p.). Group V received loperamide and kept as a positive control. Immediately afterwards, diarrhea was induced by 0.5 ml of 100µg/kg, i.p. dose of PGE_2 (Sigma Aldrich, USA). After 30 minutes, the animals were sacrificed, small intestine was removed and intestinal contents were collected and measured in a syringe. The percentage inhibition in intestinal fluid was determined by comparing the values with vehicle control.

Antimicrobial Activity: Sterile 6.0 mm diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances of MLCB at the dose of 500µg/disc. This disc, along with standard discs (Ciprofloxacin, Oxoid Ltd, UK) and control discs were placed in petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates then kept in an incubator (37°C) to allow the growth of the bacteria. The antibacterial activities of the test agents were determined by measuring the diameter of the zone of inhibition in terms of millimeter. Antimicrobial activity was tested against Staphylococcus aureus, *Escherichia coli, Pseudomonus aeruginosa, Salmonella typhi, Shigella boydii, Shigella flexneri and Shigella dysenteriae* were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) [21].

Statistical Analysis: All values were expressed as the mean ± standard error of the mean (SEM) of three replicate experiments and were analyzed using the GraphPad program (GraphPad, San Diego, CA, USA). The analysis was performed by using student's t test. $p<0.001$ and $p<0.05$ were considered to be statistically significant.

RESULTS

Total Phenolic and Flavonoid Contents: The total extractable phenolic contents of MLCB were 93.03 ± 0.21 mg/g plant extract (in GAE). In case of flavonoid, MDIB also displayed the highest flavonoid content (105.84 ± 0.19 mg/g plant extract in QA). (Table 1)

Acute Toxicity Studies: Methanolic extract of MLCB (100 - 1000 mg/kg, body weight) given orally did not cause any death in the different dose groups. The LD_{50} values for oral administration of the plant extracts were found to be greater than 1000 mg/kg in both cases.

Effect on Castor Oil-Induced Diarrhea: The extracts significantly reduced the number of diarrheal episodes in a dose dependent manner when compared with the untreated controls. At 200 mg/kg doses, MLCB showed significant ($p<0.05$) 68.86% reduction in the number of fecal episodes, whereas loperamide offered 89.14% protection (Table 2).

Effect on Magnesium Sulphate-induced Diarrhea: MLCB extracts exhibited significant antidiarrheal activity against magnesium sulphate-induced diarrhea (Table 3). The extracts at both dose levels significantly ($p< 0.05$) reduced the extent of diarrhea and also notably delayed the onset of diarrhea in a dose dependent manner.

Table 1: Yield, total amount of plant phenolic compounds and flavonoids of methanolic extract of *Lannea coromandelica* bark.

Sample	Yield (%)	Total phenols mg/g plant extract (in GAE)[a]	Total flavonoids mg/g plant extract (in QA)[b]
MDIB	15.0%	93.03 ± 0.21	105.84 ± 0.19

[a]Gallic acid equivalents (GAE, mg/g of each extract) for the total phenolic content. [b]Quercetin equivalents (mg/g of each extract) for the total flavonoid content. The GAE and QA are expressed as means ± SEM of triplicate experiments.

Table 2: Effect of *Lannea coromandelica* bark extracts on castor oil-induced diarrhea in mice.

Group	Dose (mg/kg)	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 h	% inhibition of defaecation
Group I	Vehicle	19.55 ± 1.09	5/5	29.29 ± 1.68	-
Group II	100	49.07 ± 1.73*	4/5	17.42 ± 0.95*	40.52
Group III	200	74.03 ± 2.03*	3/5	9.12 ± 1.29*	68.86
Group IV	10	120 ± 0.13*	1/5	3.18 ± 1.58*	89.14

Values are presented as mean ± SEM, (n=5); *<0.05, respectively, compared to control by student's *t*-test. Group I received vehicle (1% CMC), Group II and III received MLCB 100 and 200 mg/kg p.o. respectively and Group IV received Loperamide 10 mg/kg p.o.

Table 3: Effect of *Lannea coromandelica* bark extracts on magnesium sulphate-induced diarrhea in mice.

Group	Dose (mg/kg)	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 h	% inhibition of defaecation
Group I	Vehicle	38.05 ± 1.19	5/5	25.25 ± 1.18	-
Group II	100	24.87 ± 1.03*	4/5	12.41 ± 1.05*	50.85
Group III	200	59.43 ± 1.43*	3/5	6.17 ± 0.89*	75.56
Group IV	10	100 ± 0.10*	1/5	4.08 ± 0.51*	83.84

Values are presented as mean ± SEM, (n=5); *<0.05, respectively, compared to control by student's *t*-test. Group I received vehicle (1% CMC), Group II and III received MLCB 100 and 200 mg/kg p.o. respectively and Group IV received Loperamide 10 mg/kg p.o.

Table 4: Effect of *Lannea coromandelica* bark extracts on charcoal meal stimulated gastrointestinal transit in mice.

Treatment	Dose (p.o.)	Mean intestinal length (cm)	Mean distance traveled by charcoal (cm)	% GI transit
1% Tween 80 in water	0.4 mL/mouse	64.16 ± 0.91	49.00 ± 1.08	76. 37
Atropine	0.1 mg/kg	62.20 ± 1.19	14.47 ± 0.79*	23.26*
MLCB	100 mg/kg	63.18 ± 1.41	25.06 ± 1.16*	39.66*
	200 mg/kg	62.86 ± 1.51	14.89 ± 1.02*	23.68*

Values are presented as mean ± SEM, (n=5); *<0.05, respectively, compared to control by student's *t*-test.

Table 5: Antibacterial activity of the methanolic extracts of *Lannea coromandelica* bark.

	Diameter of zone of inhibition (mm)	
Bacterial strain	Ciprofloxacin	MLCB
Staphylococcus aureus	25.03 ± 0.12	15.09 ± 0.14
Pseudomonas aeruginosa	22.13 ± 0.21	10.12 ± 0.04
Salmonella typhi	24.42 ± 0.11	11.09 ± 0.12
Shigella flexneri	27.34 ± 0.12	7.09± 0.42
Shigella dysenteriae	25.01 ± 0.11	14.02 ± 0.62
Shigella boydii	29.39 ± 0.14	NA
Escherichia coli	31.23 ± 0.18	15.59 ± 0.22

Assay was performed in triplicate and results are the mean of three values ± Standard Deviation. NA- Zone of inhibition < 5 mm consider as no activity.

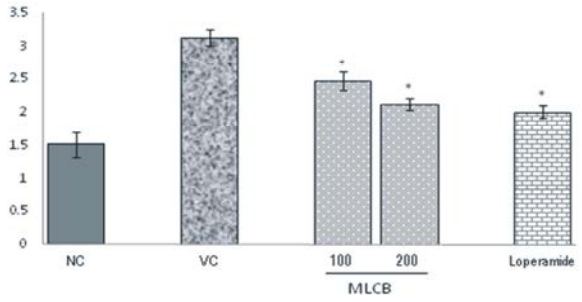

Fig. 1: Effect of the methanolic extract of *Lannea coromandelica* bark on PGE_2-induced enteropooling in mice. Values are presented as mean ± SEM, (n=5); *<0.05, respectively, compared to vehicle control by student's *t*-test. NC: Normal Control; VC: Vehicle control.

Effect on Gastrointestinal Motility: With the gastrointestinal transit experiment, the treated groups showed significant difference compared with control ($p< 0.05$). The intestinal transit of charcoal meal was 76. 37% in the control group, but at 200 mg/kg b.wt. dose of MLCB was 23.68% (Table 4).

PGE_2 - Induced Enteropooling: The plant extract reduced the intestinal fluid accumulation induced by PGE_2 in a dose dependent manner (Figure 1). At 200 mg/kg b.wt. dose, MLCB showed a good reduction (45.10%) compared with the vehicle control.

Antibacterial Activity: Table 5 expressed the antibacterial activity (zone of inhibitions) of the MLCB extracts. The MLCB extract showed significant to moderate activity against *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa and Escherichia coli.* MLCB extracts have not shown any activity against *Shigella boydii*. The highest zone of inhibition was found against *Escherichia coli* (zone of inhibition 15.59 ± 0.22 mm) followed by *Staphylococcus aureus* and *Shigella dysenteriae*.

DISCUSSION

From the ancient time, without any scientific explanation mass people are used plants or plant derived preparations to cure diarrheal disorders. Several validated studies have been proved the use of medicinal plants against diarrhea [22]. Those experimental procedures were therefore employed to judge the antidiarrheal efficacy of *Lannea coromandelica* bark in the current study.

In the present investigation, MLCB at large dose (200 mg/kg, b.wt.) exhibited significant antidiarrheal effects in one or the other experimental models. With respect to the castor oil induced diarrhea model, the results revealed that MLCB showed better protection from diarrhea in the animals as compared with vehicle control and so was the case in PGE_2 induced enteropooling. It is likely that the extracts bring out the aforementioned action either through their proabsorbtive property that

promotes faster fluid absorption in the intestine or through an anti-secretory mechanism. Our first speculation gains support from the fact that castor oil, which was used as a diarrhea inducing agent in the experimental protocol. Several mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption, activation of adenylate cyclase or mucosal cAMP mediated active secretion, stimulation of prostaglandin formation, platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil [23-25]. However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [26]. The prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings [27].

On the other hand, magnesium sulphate has been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [28]. MLCB extracts were found to improve the diarrheal condition in this model. The extracts may increase the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their antidiarrheal activity by allowing a greater time for absorption.

In the small intestinal transit test, both extracts suppressed the propulsion of charcoal marker in a dose dependent manner. This finding suggests that the extracts act on all parts of the intestine. A decrease in the motility of gut muscles increases the stay of substances in the intestine [29]. This allows better water absorbtion. It is therefore presumed that the reduction in the intestinal propulsive movement in the charcoal meal model may be due to antispasmodic properties of the extracts. Salah *et al.* [30] has reported that flavonoids inhibit the intestinal motility in experimental induced diarrhea in rats.

Flavonoids and sugars obtained from selected traditional medicinal plants in Bangladesh were reported by Rahman and Wilcock having antidiarrheal properties [31]. The flavonoids presence of these types of compounds, such as kaemferol, myricetin, apigenin and leucocyanidin in *Lannea coromandelica* is likely to contribute to its grastrointestinal effects [32]. Also some plants show antidiarrheal properties by their antimicrobial activities [33]. MLCB was shown to exhibit good antibacterial activity when tested against *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Staphylococcus aureus* and also supported to the previous study [34]. Phytoconstituents such as saponin, phenolic compounds, flavonoids and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections. In the present study this possibility is supported by the estimation of total polyphenols and flavonoids, which was found to be present in high concentration and was found to be 105.84 ± 0.19 mg equivalent of Quercetin/*g* plant extract. Moreover, Mokbel *et al.* [35] isolated various antibacterial compound viz. ß-sitosterol, malic acid, succinic acid, palmatic acid, 12-hydroxystrearic acid, glycoside, the d-malic and 12-hydroxystrearic acid. So the antibacterial activity showed by the extract may be due to the presence of those compounds.

In conclusion, the results obtained in the present study suggest that *Lannea coromandelica* bark extracts have beneficial effect in controlling the diarrhea in experimental animals. The antidiarrheal property of *Lannea coromandelica* is mediated through inhibition of hypersecretion, gastrointestinal motility and increase of gastric transit time. The *Lannea coromandelica* could be used in the treatment of diarrhea.

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