

Antidiarrheal Activity of Four Different Species of *Litsea* Available in Bangladesh

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The objective of the present study includes the evaluation of the antidiarrheal properties of the methanol extracts of *Litsea deccanensis* Gamble (MELD) bark, *Litsea lancifolia* (Roxb.) Hook. f. MELL, *Litsea glutinosa* Gamble (MELG) and *Litsea monopetala* Roxb. (MELM) leaves in Swiss albino mice. The antidiarrheal activity was evaluated by measuring percentage inhibition of diarrheal feces, total fecal output, gastrointestinal motility and by using peristaltic indices. Castor oil was used to induce diarrhea in the experimental animal. The experiments were carried out by using three different doses (100, 200, and 400 mg/kg body weight) of these four plant extracts. The number of wet feces and total weight of the feces were significantly ($p < 0.05$) and dose-dependently reduced by all the plant extracts and this effect was comparable with standard drug. MELD, MELL, MELG and MELM extracts at dose of 400 mg/kg body weight demonstrated diarrheal inhibition by 43.55%, 45.16%, 32.26% and 41.94%, respectively while it was 98.39% for the standard loperamide. Percentage (%) of fecal output for MELD, MELL, MELG and MELM extracts at the dose of 400 mg/kg were 40.14%, 62.27%, 64.06%, 46.26%, respectively. The gastrointestinal motility induced by castor oil was also reduced noticeably ($p < 0.05$) by all the plant extracts with the increasing doses. The percentage inhibition of gastrointestinal motility at the dose of 400 mg/kg were 26.26%, 33.22%, 32.36% and 22.52% for the MELD, MELL, MELG and MELM extracts respectively, while it was 27.56% for loperamide. In most cases, all the plant extracts can reduce the peristaltic indices which were comparable to control. The obtained results from this study revealed that the methanol extracts of four different species of *Litsea* found in Bangladesh may have antidiarrheal potential. It also provides the basis for the traditional use of these plants to treat diarrhea.

Keywords: Antidiarrheal, *Litsea deccanensis*, *Litsea lancifolia*, *Litsea glutinosa*, *Litsea monopetala*, mice, methanol extracts.

Diarrhea is a foremost public health issue worldwide, particularly in developing countries the global death rate is 8% among children below age 5 in 2017. In Bangladesh during 2010–12 the rate

of deaths, among children < 5 years was 4% due to acute diarrhea, 2% because of diarrhea-induced illness and 53% due to pneumonia in addition to diarrhea¹.

Diarrhea is a change in normal fecal output and is characterized by frequent passing of loose and watery stool with increased fecal volume, rate of bowel movement, abdominal pain and decreased absorption of fluid². For long years, plants with medicinal value have been used to treat various ailments counting diarrhea also³. In developing countries, most of the people rely on medicinal plants to treat diarrhea^{4,5}.

The Lauraceae family contains of nearly 55 genera and more than 2,000 species throughout the world⁶. *Litsea* genus belongs to the laurel family, Lauraceae and the plants are deciduous or evergreen shrubs or trees. There are more than 600 species widely distributed in tropical and subtropical regions including South America, North America, Asia, Australia and New Zealand. More than 407 phytochemicals of diverse varieties including sesquiterpenes, lactones, flavonoids, lignans, alkaloids etc. have been reported from *Litsea* species⁷. Most of these secondary metabolites possess significant bioactivities as antidiarrheal, antimicrobial, insecticidal, anti-HIV, antioxidant, analgesic, anti-inflammatory etc. In Bangladesh, there are few references on this genus and 11 species of *Litsea* are listed by the Ministry of Environment and Forest, Bangladesh. Among them we have selected four species for our study.

Litsea deccanensis Gamble is distributed in Chattogram, Bangladesh. In Andhra Pradesh, India *L. deccanensis* leaves are used in chest pain and the extract of this plant was studied to possess compelling antioxidant and reducing capacities and cardioprotective effect in rat models. α -humulene, α -Caryophyllene, caryophyllene epoxide, bicyclogermacrene, germacrene-3,9,11-triene; Squalene, quassin, stigmasterol, vitamin E, oleic acid and several alkaloids boldine, corytuberine, dicentrine, nordicentrine, lauroitsine, isocorydine, magnoflorine were isolated from *L. deccanensis*⁸⁻¹⁰.

Litsea lancifolia (Roxb.) Hook. f. named by Chakma tribes as Judijaylla belongs to the Lauraceae family is distributed in the south-eastern portion of Bangladesh. *L. lancifolia* root is used to treat diarrhea by Chakma tribes in Rangamati, Bangladesh¹¹. *L. lancifolia* exhibited promising anti-diabetic effect and found nontoxic to 3T3L1 cells¹². Bulbul *et al.* (2020) reported *L. lancifolia* to have anti-oxidant, analgesic,

antimicrobial, CNS depressant and hypoglycemic activities¹³. Sulaiman *et al.* (2011) reported the alkaloids namely lancifoliaine, boldine, norboldine, actindaphnine, etc in the bark of this plant¹⁴. Li *et al.* (2008) reported alkaloids litseferine, juziphine, phanostenine and a steroid β -sitosterol. Aristotetralone, dehydrodiisoeugenol, 4'-methylenedioxyflavan-3-ol, 5,7-dimethoxy-3', β -hydroxybenzoic acid, β -sitosterol, vanillin and dihydrodehydrodiconiferylalcohol were also isolated from *L. lancifolia*¹⁵⁻¹⁶.

Litsea glutinosa Gamble (Lauraceae) is a medium-sized tree, growing in the forest of Chattogram and Sylhet districts in Bangladesh¹⁷. In Bangladesh, China, India, Myanmar, Sri Lanka, Malaysia *L. glutinosa* bark, leaves, roots and fruits are used for diarrhea, abscess and traumatic injury while the essential seed oil is used traditionally for rheumatism¹⁸. Previously *L. glutinosa* was evaluated for its thrombolytic, analgesic, anti-inflammatory, antipyretic, antibacterial, anti-diabetic (type II), antioxidant, hepatoprotective activities¹⁹⁻²¹. Several alkaloids such as isoboldine, laureliptine, Liriodenine, actinodaphnine, n-methylactinodaphnine, laurotetanine, n-methylaurotetanine, lauroitsine, boldine, litseferine, litsine and glutinosine A were previously reported in *L. glutinosa*²²⁻²⁴. Some flavonoids such as 2',5,7-trihydroxy-6-methoxyflavone 2'-O-beta-D-glucopyranoside²⁵; sesquiterpenes like α -Caryophyllene, Caryophyllene oxide and monoterpenes like (E)- α -Ocimene, (Z)- α -Ocimene were isolated from *L. glutinosa*²⁶.

Litsea monopetala Roxb. Pers. belongs to the Lauraceae family and spreads in the hill tract forests, Sal forests and in the village areas of Bangladesh. In Bangladesh, Nepal, Myanmar, India, China *L. monopetala* is traditionally used in treating fracture and dislocation, skin disease, gonorrhoea, diarrhea and also to cure pains²⁷. This plant species was also reported for its antioxidant, antimicrobial, analgesic, hypoglycemic, CNS depressant and antidiarrheal activities²⁷⁻²⁹. Chalcone & its derivatives and eugenol²⁷, caryophyllene oxide, α -caryophyllene alcohol, humulene oxide, tricosane and pentacosane in the flower; capric acid, nonanol and decanal in fruit and myristic acid, tridecanol, tridecanal and tetradecanal in bark were also reported from *L. monopetala*³⁰.

Considering the traditional uses of above-

mentioned plants of *Litsea* species in the treatment of diarrhea as well as few evidence-based reports of this effect, our present study was aimed to evaluate their antidiarrheal effect in animal.

METHODS

Collection of plant materials and preparation of plant extracts

The bark of *L. deccanensis* (DACB-35517), the leaves of *L. lancifolia* (DACB-35164), *L. glutinosa* (DACB-37904) and *L. monopetala* (DACB-38437) were collected from Chattogram hill track, Bangladesh. All the plant samples were identified by a taxonomist at Bangladesh National Herbarium, Mirpur-1, Dhaka-1216, Bangladesh.

After collection, the plant samples were washed gently with tap water and then shade dried for several days, and finally crushed to granular powder. The grounded plant materials (800 g to 1000 g) for each plant were drenched in methanol in flat bottom containers at room temperature for seven days with occasional shaking and stirring. The extracts were then filtered twice by using cotton mass followed by filter papers (Whatman No. 1). A rotary evaporator (Heidolph, Germany) was used to concentrate the filtrates at reduced pressure and temperature. All the crude extracts were evaporated to dryness and kept in a moisture free cold environment for further analysis.

Experimental animals

Swiss albino mice of either sex (Weight: 30-40 g; Age: 6-8 weeks) were procured from Jahangirnagar University, Dhaka, Bangladesh for the experiment. The animals were accommodated in standard mice cages at 25°C and a proper day/night cycle was maintained. Before the beginning of the experiment, the animals were adapted for seven days for acclimatization. In the complete study the animals were handled according to the guidelines by the National Research Council, Washington, DC³¹. Those guidelines were accepted by the committee on ethical compliance in research (SEU/Pharm/CECR/103/2021) of Southeast University.

Acute oral toxicity test

The toxicity study after oral administration of the plant extracts was conducted according to OECD 420 Standard³². Swiss albino mice (Female; weight: 30-40 g; Age: 4-6 weeks) were taken for

the study. This experiment was started with one fasted mouse that was given plant extract orally at a dose of 2000 mg/kg body weight and noticed for any toxic influence of the extract like increased motor activity, coma or death. As there was no death within 24 h then other four mice were treated with the same dose for each extract. Following the administration of four extracts, the treated animals were observed for toxic and behavioural effects every day for about 14 sequential days.

Experimental design

Extracts coding for different doses

Three different doses (100, 200 and 400 mg/kg) of the methanol extract of *L. deccanensis* were coded as MELD_1, MELD_2 and MELD_3, respectively; for *L. lancifolia* the codes were MELL_1, MELL_2 and MELL_3; for *L. glutinosa* the codes were MELG_1, MELG_2 and MELG_3 and for *L. monopetala* they were MELM_1, MELM_2 and MELM_3, respectively.

Animal grouping and dosing

Swiss albino mice were allocated into five groups containing five mice in each group as follows:

Group I: Control, specified to administer only vehicle (10 ml/kg, distilled water)

Group II: Standard control, specified to administer standard Loperamide (3 mg/kg body weight)

Group III: Treatment group, specified to administer the plant extract (100 mg/kg body weight)

Group III: Treatment group, specified to administer the plant extract (200 mg/kg body weight)

Group III: Treatment group, specified to administer the plant extract (400 mg/kg body weight)

Antidiarrheal activity test

Castor oil-induced diarrhea

Antidiarrheal activity was evaluated by castor oil-induced diarrheal method in mice³³. The animals were divided into negative control, positive or standard group, and test groups, as discussed in the animal grouping and dosing section. After 60 min of administration of three different doses (100, 200 and 400 mg/kg body weight) of four different plant extracts (MELM, MELL, MELG and MELD) and standard Loperamide (3 mg/kg body weight), 0.5 ml castor oil was given orally to every mouse to induce diarrhea. The experimental animals were then retained separately in a plastic cage with the floor on which a white paper was placed to note the number of wet stools (diarrheal stool), total number and total weight of the faecal output

for consequent four hours. Every hour the white paper was changed. Then, diarrheal inhibition (% inhibition of wet defecation) and the percentage of faecal output (% FOP) were calculated by the following equations:

$$1. \text{Diarrheal inhibition (\% inhibition of wet defecation)} = \frac{WD_c - WD_T}{WD_c} \times 100\%$$

Where, WD_c = Mean wet defecation of control, WD_T = Mean wet defecation of standard drug/ test samples.

$$2. \% \text{ Faecal output (FOP)} = \frac{FW_T}{FW_c} \times 100\%$$

Where, FW_T = Mean faecal weight of each treatment group; FW_c = Mean faecal weight of control group.

Gastrointestinal motility test By using barium sulphate meal

This study was completed conferring the reported method of Chatterjee (1993), updated by Mazumdar *et al.* (2015) and investigated the effect of the plant extracts on the gastrointestinal motility induced by castor oil^{34,35}. Experimental mice fasted for 18 h were grouped as negative control, positive control and treatment group, as discussed in the previous section. One hour after treatment with standard loperamide (3 mg/kg body weight) and

three different doses (100, 200 and 400 mg/kg body weight respectively) of four different plant extracts, 0.5 ml of castor oil was administered by oral gavage to initiate diarrhea in mice. Following one hour of castor oil administration, all mice were given 1 ml of 5% barium sulphate suspension by oral gavage. White barium sulphate suspension was used in this method, because it is easily visible in normal light which can help for the measurement of the distance travelled by the barium sulphate meal of the intestine. After 30 min of barium sulphate administration, all the animals were sacrificed and the small intestine of each mouse was isolated. Then the entire length of the intestine and the intestinal length travelled by the barium sulphate meal were measured by using centimetre scale.

By using the succeeding formula, the percentage of inhibition of the gastrointestinal motility and peristaltic index were calculated.

$$1. \% \text{ inhibition of the gastrointestinal motility} = \frac{D_c - D_T}{D_c} \times 100\%$$

Where, D_c = Mean distance travelled by the control; D_T = Mean distance travelled by the test group.

$$2. \text{Peristalsis Index} = \frac{\text{Distance travelled by barium sulphate meal}}{\text{Length of small intestine}} \times 100\%$$

Table 1. Effect of the methanol extracts of *L. monopetala*, *L. lancifolia*, *L. glutinosa* and *L. deccanensis* on castor oil-induced diarrhea in mice

Treatment Groups	Dose (mg/kg, p.o)	Total number of feces	Total number of wet feces	% inhibition of wet defecation	% of fecal output
Control	-	22.5±1.85	15.5±2.02		
Loperamide	3	2±0.71*	0.25±0.25*	98.39	12.28
MELD_1	100	12.25±2.06*	9.75±1.60	37.10	46.62
MELD_2	200	10.25±1.70*	9.25±1.89	40.32	39.86
MELD_3	400	9.75±1.49*	8.75±1.65*	43.55	40.14
MELL_1	100	16.25±1.03	9.5±0.28	38.71	74.02
MELL_2	200	16.75±1.32	9.25±1.11	40.32	70.11
MELL_3	400	14.75±0.85*	8.5±0.5*	45.16	62.27
MELG_1	100	19.5±1.041	13.25±1.32	14.52	81.14
MELG_2	200	17.5±0.65	13±1.41	16.13	77.58
MELG_3	400	15.5±3.66*	10.5±3.23	32.26	64.06
MELM_1	100	17±0.71	10.25±0.95	33.87	55.87
MELM_2	200	14±1.0*	9.75±0.63	37.10	52.31
MELM_3	400	14±1.68*	9±0.71*	41.94	46.26

All values are stated as mean ± SEM (n = 5); One way ANOVA then a Tukey post hoc test was carried out for data analysis. Here, * values are statistically significant at P<0.05

Statistical analysis

The Graph Pad Prism 8 was used to perform the statistical analysis and all values are expressed as mean \pm SEM (n=5). A one-way analysis of variance (ANOVA) followed by a Dunnett test was performed to compare multiple groups. Values were measured statistically significant at $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS

Results acute oral toxicity test

After administration of a single oral dose of 2000 mg/kg body weight for all the plant extracts, the animals were observed for 14 days and found no significant toxic effects and death incidence. Water and food intake by the animals were normal during this observation period. So, it can be concluded that MELD, MELL, MELG and MELM have a broader safety margin and their LD₅₀ value will be more than 2000 mg/kg.

Effect of test extracts on castor oil-induced diarrhea

In this experiment, the tested plant extracts MELD, MELL, MELG and MELM exhibited a dose-dependent reduction in the number

of wet feces (diarrheal stool), total number and total weight of the feces and the results were significant ($p<0.05$) for all the plant extracts at a dose of 400 mg/kg, as compared with control. Castor oil-induced diarrheal feces were noticeably reduced by all the extracts and the maximum diarrheal inhibition was observed for MELD, MELL, MELG and MELM extracts at 400 mg/kg and that were 43.55%, 45.16%, 32.26% and 41.94%, respectively, while it was 98.39% for the standard Loperamide. Though all the extracts exhibited a similar effect, the order for the inhibition of wet defecation was MELL>MELD>MELM>MELG. Percentage (%) of fecal output for MELD, MELL, MELG and MELM extracts at 400 mg/kg were 40.14%, 62.27%, 64.06% and 46.26%, respectively and the order of the plant extracts for fecal output was MELD>MELM>MELL>MELG (Table 1).

Effects of test extracts on gastrointestinal motility

In this test, all the extracts (MELD, MELL, MELG and MELM) also exhibited significant ($p<0.05$) and a dose-dependent reduction in the gastrointestinal motility induced by castor oil. The plant extracts MELL, MELG, MELD and MELM at the dose of 400 mg/kg

Table 2. Effect of the methanol extracts of *L. monopetala*, *L. lancifolia*, *L. glutinosa* and *L. deccanensis* on gastrointestinal motility in mice

Treatment Groups	Dose (mg/kg, p.o)	Total Length (cm) of Intestine	Distance (cm) traveled by Barium Sulphate	% of Inhibition	Peristalsis Index (%)
Control	-	53.1 \pm 7.12	47.943 \pm 4.06		90.3
Loperamide	3	52.070 \pm 1.80	34.73 \pm 1.59	27.56	66.7
MELD_1	100	47.158 \pm 3.31	44.45 \pm 1.80	7.29	94.3
MELD_2	200	47.568 \pm 2.98	37.748 \pm 1.66	21.26	79.4
MELD_3	400	59.860 \pm 2.87	35.353 \pm 5.06	26.26	59.1
MELL_1	100	48.013 \pm 2.10	35.268 \pm 6.14	26.44	73.5
MELL_2	200	53.658 \pm 2.10	32.385 \pm 9.53	32.45	60.4
MELL_3	400	50.8 \pm 1.56	32.018 \pm 7.42	33.22	63.0
MELG_1	100	50.165 \pm 1.10	35.242 \pm 2.66	26.49	70.3
MELG_2	200	51.435 \pm 2.67	32.933 \pm 2.31	31.31	64.0
MELG_3	400	54.928 \pm 3.38	32.428 \pm 6.66	32.36	59.0
MELM_1	100	48.135 \pm 1.74	41.225 \pm 2.39	14.01	85.6
MELM_2	200	48.955 \pm 0.62	39.688 \pm 3.57	17.22	81.1
MELM_3	400	47.05 \pm 1.29	37.148 \pm 3.64	22.52	79.0

All values are stated as mean \pm SEM (n = 5); One way ANOVA then a Tukey post hoc test was carried out for data analysis. Here, * values are statistically significant at $P<0.05$

showed maximum influence by 33.22%, 32.36%, 26.26% and 22.52%, respectively whereas standard loperamide (3 mg/kg body weight) treated group showed a greater antimotility effect by 27.56% than all of the plant extracts. The order of the plant extracts for inhibition of intestinal motility was MELL>MELG>MELD>MELM. The peristaltic indices were reduced by the plant extracts at all doses, compared to control except MELD at dose of 100 mg/kg body weight (Tables 2).

DISCUSSION

Traditionally many medicinal plants having antidiarrheal effect are used in our folk medicine for the management of diarrheal disease. It is therefore imperative to find out available natural drugs as alternatives to commonly used synthetic antidiarrheal drugs, which are associated with serious adverse effects. In Bangladesh, a range of medicinal plants with antidiarrheal properties has been widely used by common people³⁶.

In our current study the antidiarrheal properties of the methanol extract of four different species of *Litsea*, i.e., *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) was determined and the results of our study clearly portrays significant antidiarrheal properties in mice model. All the plant extracts showed significant ($p<0.05$) and a dose-dependent inhibition of castor oil-induced diarrhea and gastrointestinal motility in animal. This inhibitory effect justifies the folkloric use of *L. lancifolia*, *L. glutinosa* and *L. monopetala* in the treatment of diarrhea. The antidiarrheal effect of *L. monopetala* observed in this study has supported the previous reports²⁸, while for *L. lancifolia*, *L. glutinosa* and *L. monopetala*, this is the first report of their antidiarrheal activity.

Castor oil-induced diarrheal model is a common technique which is used to evaluate the antidiarrheal activity of plant extracts in animal. Castor oil produces diarrhea in mice by changing the permeability of electrolytes through the intestinal mucous membrane³⁷⁻³⁸. Ricinoleic acid, an active metabolite of castor oil, induces mucosal irritation and inflammation via augmented secretion of prostaglandins which ultimately increases GI motility and secretion.

In this experiment, the methanol extracts

of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* effectively exhibited antidiarrheal action by the inhibition of castor oil-induced prostaglandin synthesis. The antidiarrheal activity might also be due to inhibition of ricinoleic acid secretion, resulting in the stimulation of Na⁺, K⁺ ATPase activity which enhances absorption of electrolytes in the intestinal mucosa³⁹. It can be presumed that this antidiarrheal effect of the tested plant extracts is due to their antisecretory and antimotility properties, a similar mechanism produced by loperamide, a commonly used antidiarrheal drug. So, the reduced intestinal motility and fluid accumulation within the gastrointestinal tract of the treated animal by the plant extracts may be mediated through the similar mechanism by the standard loperamide.

This antidiarrheal effect could probably be associated with the occurrence of phytochemicals such as terpenoids, tannins and flavonoids present in the plant extract, MELD, MELL, MELG and MELM which are shown to inhibit the prostaglandin release and intestinal absorption of electrolyte. Many studies have confirmed several phytochemicals in medicinal plants to produce antidiarrheal activity by increasing antispasmodic effects, suppressing gut motility, delaying intestinal transit, stimulating water adsorption or reducing electrolyte secretion⁴⁰. Phytochemicals such as flavonoids and tannins are reported to exhibit antidiarrheal activity by stimulating electrolyte and water reabsorption from small intestine⁴¹⁻⁴². Four of our studied plants have been reported previously to have many of these phytochemicals predominantly alkaloids^{14-15, 22-25}.

CONCLUSION

The obtained results from this study revealed that the methanol extracts of four different species of *Litsea* found in Bangladesh have remarkable antidiarrheal potential. All these four plant extracts may produce the antidiarrheal activity by stimulating the reabsorption of electrolyte and water, and by checking intestinal motility as all of these plants are rich sources of alkaloids, flavonoids and tannins. This result also provides the basis for the traditional uses of these plant species as antidiarrheal agent. However, more detailed phytochemical analysis will be necessary

to identify the active constituents responsible for the pharmacological activities of *Litsea* species.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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