Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(1):27-34
(http://www.derpharmachemica.com/archive.html)

Comparative Anti-dirrhoeal and Antimicrobial Activities of Methanol Extract of *Leea indica* (Burm. f.) Merr. and *Leea macrophylla* Roxb. Ex. Hornem (Fam. Vitaceae) and Four Bangladeshi Market Preparations

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ABSTRACT

Both Leea indica and Leea macrophylla are belonging to Leeaceae family. The aim of the present study was to detect different chemical groups of the methanol leaf extract of the plants (L. indica & L. macrophylla) and to compare the anti-diarrheal and antimicrobial activity of these extracts with four established market preparations. Phytochemical screening of extracts of L. indica, L. macrophylla confirmed the presence of alkaloids, glycosides, steroids, tannins, flavonoids, reducing sugars, and gums. In anti-diarrheal activity test, the methanol extracts of the L. indica and L. macrophylla at the doses of 500 mg/kg and 250 mg/kg reduced the total number of stool in the mice to a considerable extent as well as increased the latency period in comparison to the control groups and the result was statistically significant but less in extent than market preparations. In the antibacterial activity test, it was observed that both the plant extracts did not produce significant zone of inhibition against all the pathogenic bacteria. On the other hand, in the anti-fungal activity test, it was observed that the extracts and market preparation showed significant antifungal activity against fungal species compared to fluconazole as a standard. In conclusion, methanol extracts of L. indica and L. macrophylla indicate that the plants contain effective bioactive compounds that can be used for the treatment of diarrheal and microbial diseases.

Keywords: *Leea indica, Leea macrophylla*, Anti-diarrheal, and Antimicrobial

INTRODUCTION

Leea indica (Burm.f.) Merr. (Family: Leeaceae), an evergreen perennial shrub with stout, soft wooded, glabrous stems native to tropical Asia, Australasia, Pacific and grown habitually in Bangladesh, India, China, Bhutan and Malaysia [1]. Traditionally, the whole plant is used to mitigate headache, body pain, and skin complaints [2]. Specifically, leaves and roots of *L. indica* are traditionally used for the management of cancer, diabetes, diarrhea, dysentery, spasm and skin diseases [3]. Combined root paste of this plant along with the root of *Oreocnide integrifolia* and *Cissus repens* in bubo and boils are prescribed by Marma tribes of Chittagong Hill Tracts, Bangladesh [4]. Previous pharmacological studies reported that it possesses antioxidant [3,5,6], analgesic [7], CNS depression [8], phosphodiesterase and nitric oxide synthase inhibitory [9], hepatoprotective [2], cytotoxic [3,4] anticancer [1,10] activities.

Leea macrophylla (Roxb.) is locally identified as Hathikana or Hatikana. It is belonging to Leeaceae family, a herb or herbaceous shrub with a very big sized leaf like an elephant ear [11]. The plant is indigenous to North-Eastern India; though, it is distributed to the relatively hotter parts of India, central and eastern Nepal, Bhutan, China, Myanmar, Thailand, Cambodia, and Laos [12]. In many areas of Bangladesh such as Rajshahi, Jessore, And Natore are noteworthy for the habitat of *L. macrophylla* [13]. Regarding the ethnobotanical survey, this plant shows some important therapeutic indications in cancer, dysentery, body-ache, and sexual disability [14]. Traditionally, it has some other uses for tonsillitis, tetanus, nephrolithiasis, rheumatism, arthritis, snake bites, sore, pain, and blood effusion [15,16]. This plant is widely used by the ayurvedic physicians in the preparation of seasonal tonic modaka preparation [11]. The juice from the leaf is recognized as local anti-inflammatory agent and used in boils, arthritis, gout, and rheumatism [17]. Seed extract of this plant showed the presence of carbohydrate, protein, glycosides, phenolics, and saponin. The

extracts also displayed antifungal activities against *Candida albicans* except the extract n-hexane [18]. Several literatures revealed anti-inflammatory and analgesic [19], antinociceptive and cytotoxic [20], and urolithiatic [21] effects of leaf extract of this plant. Root extract shows antioxidant, and antibacterial activites [22].

Therefore taking these into consideration, the overall aim of the present study is to detect different chemical groups of the methanol leaf extract of the plants (*L. indica and L. macrophylla*) and to compare the anti-diarrheal, and antimicrobial activity with four established market products.

MATERIALS AND METHODS

Plant material

For the investigation, the fresh leaves of *Leea indica* and *Leea macrophylla* were collected from the hill of Khamarpara, Rangamati, Chittagong, Bangladesh. The extraneous, undesired substances from the plant material were removed at two stages of processing of it. At first the rotten leaves, stems etc. were removed by hands immediately after collection of the leaves. Again, the soil was removed by sieving through a net aided by a flow of air from an electric fan before the plant materials are dried.

The plants *L. indica* and *L. macrophylla* were selected based on its medicinal uses. The traditional practitioners called as "Kabiraj" and the tribal people of hill tracts were the main source of reliable information about the traditional uses of this plant. Taxonomical identification of this plant was made by the experts of Bangladesh Forest Research Institute Herbarium (BFRIH), Chittagong.

Extraction of plant material

The collected leaves were separated from undesirable materials (plant parts or dust) or plants and were shed-dried (35-50ºC). The leaves were ground into coarse powder with the help of a grinder. The powder were stored in airtight containers and kept in a cool, dark and dry place until extraction commenced.

The extracts were weighed separately with the help of electronic and digital balance.

Then the yield was determined by using the following formula:

Yield=[wt of crude extract (g)/wt of initial powder taken (g)] × 100

The amount of crude extracts *of L. indica*, *L. macrophylla* from hot extraction was 10 g respectively.

Yield=[10 g/150 g] × 100=6.67%

Animals

Young Swiss-albino mice, average weight of 18-25 g of either sex were employed in the experiment. The mice were collected from the Animal Research Branch of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh. The mice were kept separately in wooded cages having dimension of 15 × 10 × 8 inch. Soft wood shavings were placed in the cages for housing of the mice. The mice were housed in a well-ventilated air and lightened house.

Phytochemical investigation

The extracts were subjected to preliminary phytochemical investigation. Small quantity of freshly prepared methanol extract of *L. indica*, *L. macrophylla* were subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals such as alkaloids, glycosides, steroids, tannins, flavonoids, saponins, reducing sugars, gums & mucilage using the standard methods [23-29].

In vitro anti-dirrhoeal activity

Preparation of samples for the test, standard and control groups

In the present work, 500 mg/kg and 250 mg/kg doses were selected for plant species and for this, 500 and 250 mg of plant extracts were dissolved respectively in small amount of Tween-80 then the final volumes were adjusted by distilled water.

For reference standard group, 3 mg of Loperamide was dissolved in 10 ml distilled water and so for the four market preparations. For control group small amount of (1%) Tween-80 was mixed with distilled water and final volume was adjusted to make 10 ml.

Experimental design

The method, described by Shoba and Thomas [30] was followed for this study. The animals were all screened initially by giving 0.4 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were then, divided into control, positive control and two test groups containing three mice in each group. Control group received vehicle (1% Tween-80 in water) at a dose of 10 ml/kg body weight orally. The positive control group received Loperamide at the dose of 3 mg/kg orally and same dose were taken for the four market preparation; test groups received the methanol extract of *L. indica* and *L. macrophylla* respectively; at the dose of 500 mg/kg and 250 mg/kg body weight orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhea was induced by oral administration of 0.4 ml castor oil to each mouse, 30 mins after the above treatments. During the observation period (4 hrs), the total latency periods (first diarrheal stool after the administration of castor oil) and the number of diarrheic feces excreted by the animals were recorded. A numerical

score based on stool consistency was assigned (normal stool=1 and watery stool=2).

Determination of in vitro anti-diarrhoeal activity

Anti-diarrheal episode was determined by evaluating the latency period and diarrhoeal frequency by counting the fecal time and number of the test groups in comparison to the control and standard drug Loperamide groups.

***In vitro* antimicrobial activity**

Antimicrobial screening

The antibacterial and antifungal activities of the crude extracts were evaluated by the disc diffusion method Aboaba and Efuwape, [31] against 4 Gram positive and 6 Gram negative pathogenic bacteria and 7 fungi using ciprofloxacin and fluconazole as standards. All the microbial species were collected from the Microbiology Lab of Department of Pharmacy, Southern University Bangladesh, and Chittagong, Bangladesh. The antimicrobial activity of the test agents was expressed by measuring the diameter of zone of inhibition expressed in mm. The experiments were carried out in triplicate.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of all the extract was determined by the serial dilution technique [32] in nutrient broth medium, containing graded concentration of the plant extracts and inoculated test organisms.

RESULTS AND DISCUSSION

Phytochemical investigation

Phytochemical investigation of the extracts of leaves of *L. indica* and *L. macrophylla* confirmed the presence of alkaloids, glycosides, steroids, tannins, flavonoids, reducing sugars, and gums (Table 1).

***In vitro* antidirrhoeal activity**

Diarrhea, which could be infectious or non-infectious, is one of the principal causes of death, particularly in the malnourished infants [33]. In order to combat the problems of diarrhoea globally, the World Health Organization in its Diarrhoeal Disease Control Programme has given a special emphasis on the use of traditional folklore medicines in the control and management of diarrhea [34]. According to the antidirrhoeal activity, the extracts as well as market preparation shoed significant activity compared with control. Previous studies have supported that tannins, flavonoids, reducing sugars/glycosides among others have potent antidiarrhoeal and antidysentery activity [35]. Flavonoids have also been shown to have inhibitory actions on intestinal motility [36].

In the castor oil induced diarrheal mice, the methanol extracts of the *L. indica* and *L. macrophylla* at the doses of 500 mg/kg and 250 mg/kg reduced the total number of stool in the mice to a considerable extent as well as increased the latency period of defecation in comparison to the control groups and the result was statistically significant. The methanol extract of *L.*

Table 1: Chemical group test for the crude methanol extracts

Examination	Name of the test	Consequences	
		MELI	MELM
Alkaloids	Mayer's test	+	+
	Dragendorff's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
	Tannic acid test	-	-
Glycosides	Salkowski test	+	+
	Libermann-burchared test	+	+
Steroids	Salkowski test	+	+
	Libermann-burchared test	+	+
Tannins	Ferric chloride test	+	-
	Potassium dichromate test	+	-
	Keller-Killiani test	-	-
Flavonoids	Conc. HCl and alcoholic test	+	+
Saponins	Shake test (aq. solution)	-	-
Reducing sugars	Fehling's test	+	+
	Benedict's test	+	+
Gums	Molisch's test	+	+
Amides	NaOH test	-	-

+ indicates the presence of the groups/chemicals tested for
- indicates the absence of the groups/chemicals tested for
MELI indicates Methanolic extract of *Leea indica*
MELM indicates Methanolic extract of *Leea macrophylla.*

indica significantly increases latency (74 min) which was close to the reference standard, Loperamide (85 min). On the other hand, the methanol extract of *L. macrophylla* reduced the number of stool secretion significantly than the other crude extract compared to the standard (Figures 1 and 2).

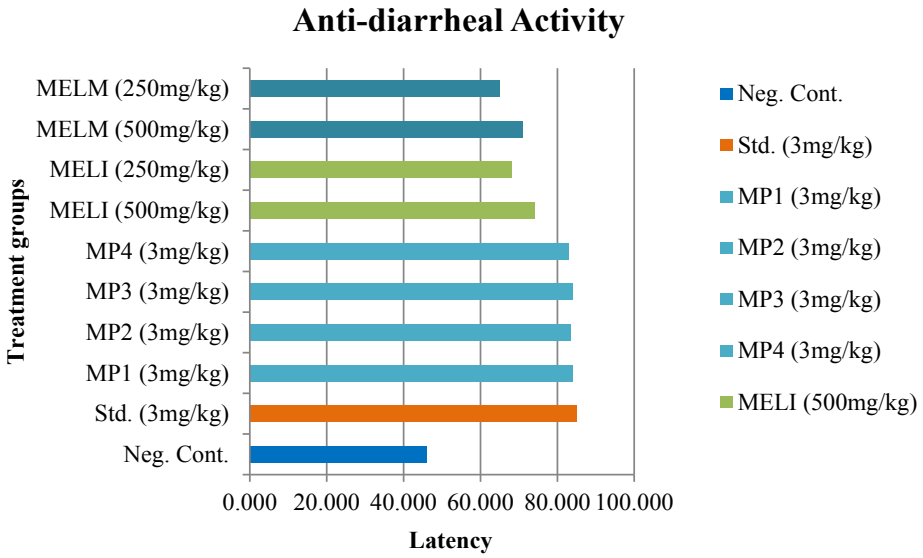

Figure 1: The latency period of different treatment groups on castor oil induced diarrheal mice

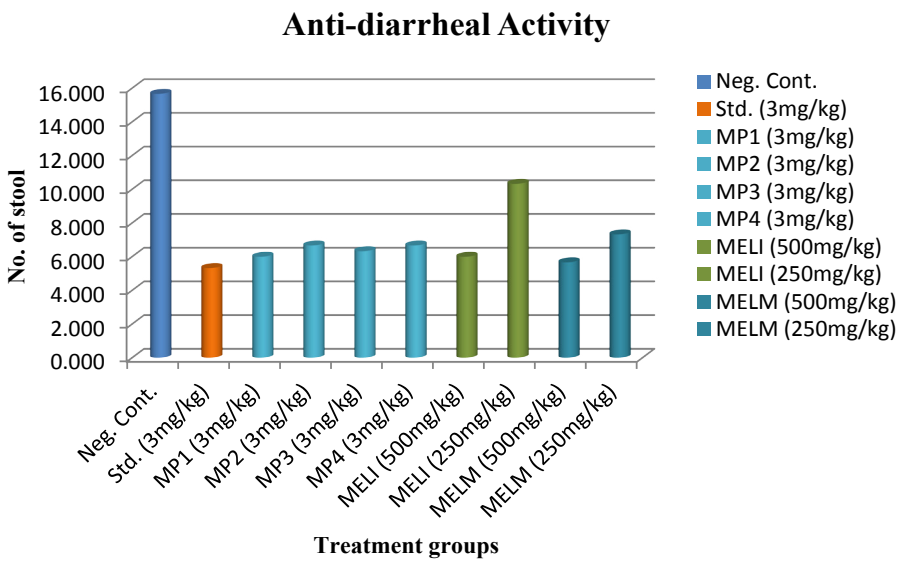

Figure 2: Number of stool secreted by different treatment groups on castor oil induced diarrheal mice

***In vivo* antimicrobial activity**

An infectious disease is a disease that is caused by the invasion of a host by agents whose activities harm the host's tissues (that is, they cause *disease*) and can be transmitted to other individuals (that is, they are infectious). Microorganisms that are capable of causing disease are called pathogens [37,38]. Infectious diseases are the leading cause of death worldwide. Bacteria and fungi are two of the five major types of infectious agents [38].

According to the antimicrobial activity test, methanol extract of *L. indica* and *L. macrophylla* and market preparations were evaluated against four (04) gram-positive and six (06) gram-negative pathogenic bacteria using erythromycin as standards. The plant extracts and market preparations exhibited very significant antibacterial activity compared to erythromycin as a standard against all the tested microorganisms. In the antibacterial sensitivity test, it was observed that both plant extracts did not produced significant zone of inhibition against all the pathogenic bacteria. The zone of inhibition was found within the range of 10.00 mm to 20.67 mm.

At the dose of 500 µg/disc the highest zone of inhibition was produced by *L. indica* (20.67 mm) against *V. cholerae* then followed by 18.00, 14.50, 14.00, 12.00, 11.00, 10.50 and 10.00 mm against *S. dysenteriae, S. sonnei, S. aureus, B. subtilis, P. aeruginosa* and *B. megateriuum* respectively. But no inhibition produced against the *E. coli* and *S. typhi* (Table 2).

Whereas at the dose of 500 µg/disc the highest zone of inhibition was produced by *L. macrophylla* (16.30 mm) against *B. cereus*. Then followed by 15.00, 13.50, 11.00, 11.00, 10.50, and 10.00 mm against *S. dysenteriae, S. aureus, B. megateriuum, V. cholera,*

Table 2: Tabulation of anti-bacterial activity (total zone of inhibition) by treatment groups

Microorganisms (Bacteria)	Zone of inhibition (MZI±SD) mm							
	Control Methanol (10 µl/disc)	Standard (Erythromycin) (30 µg/10 µl)	MP_1	MP_2	MP_3	MP_4	MELI	MELM
			(30 µg/10 µl)				(500 µg/10 µl)	
Gram positive species								
Bacillus subtilis	-	25.33 ± 0.58	24.00 ± 1.00	24.00 ± 1.00	23.33 ± 1.53	23.00 ± 1.00	12.00 ± 1.15	10.00 ± 1.15
Bacillus megateriuum	-	27.33 ± 2.08	25.67 ± 1.53	25.00 ± 0.58	24.00 ± 0.58	24.33 ± 1.00	10.00 ± 1.00	11.00 ± 1.00
Bacillus cereus	-	30.00 ± 1.73	28.00 ± 1.00	28.33 ± 1.00	27.00 ± 0.58	26.33 ± 1.00	11.00 ± 1.00	16.30 ± 1.20
Staphylococcus aureus	-	45.33 ± 0.58	43.50 ± 1.00	44.00 ± 1.53	43.33 ± 1.53	42.00 ± 0.58	14.00 ± 1.00	13.50 ± 0.60
Gram negative species								
Escherichia coli	-	41.00 ± 0.58	38.00 ± 1.00	38.00 ± 1.00	37.33 ± 1.00	37.00 ± 1.53	Ni	10.00 ± 1.53
Pseudomonas aeruginosa	-	25.67 ± 1.58	24.00 ± 1.53	24.50 ± 0.58	23.50 ± 1.63	23.33 ± 1.53	10.50 ± 1.00	Ni
Shigella dysenteriae	-	30.33 ± 0.58	28.67 ± 1.53	28.00 ± 1.00	27.33 ± 1.00	27.00 ± 0.58	18.00 ± 1.00	15.00 ± 1.00
Shigella sonnei sonnei	-	25.00 ± 1.00	24.00 ± 1.00	23.33 ± 1.17	22.00 ± 1.08	21.33 ± 1.15	14.50 ± 0.58	12.50 ± 1.20
Salmonella typhi	-	30.00 ± 0.58	28.00± 0.58	28.33± 0.58	23.00± 0.58	25.50± 1.00	Ni	Ni
Vibrio cholera	-	25.33 ± 2.08	22.67 ± 1.15	22.33 ± 1.53	22.00 ± 1.00	21.50 ± 1.00	20.67 ± 0.58	11.00 ± 0.58

SD = Standard Deviation, MZI: Mean zone of inhibition (mm); zone of inhibitions under 8 mm were considered as less active and were discarded. Ni=No inhibition, MP = Market Product, MELI = Methanol extract of *L. indica*, MELM = methanol extract of *L. macrophylla*.

Table 3: Tabulation of anti-fungal activity (total zone of inhibition) by the treatment group

Microorganisms (Fungi)	Zone of inhibition (MZI±SD) mm							
	Control Methanol (10 µl/disc)	Standard (Fluconazole) (30 µg/10 µl)	MP_1	MP_2	MP_3	MP_4	MELI	MELM
				(30 µg/10 µl)			(500 µg/10 µl)	
Aspergillus niger	-	16.33 ± 0.58	14.00 ± 0.58	14.33± 0.58	15.00 ± 1.00	14.67 ± 1.00	12.00 ± 1.53	10.00 ± 0.58
Blastomyces dermatitidis	-	14.00 ± 1.00	12.00 ± 1.00	12.33 ± 0.58	13.00± 0.58	12.00 ± 0.58	11.00 ± 1.00	10.33 ± 1.53
Candida albicans	-	15.00 ± 1.00	13.00 ± 2.00	14.00 ± 1.00	14.00 ± 0.58	13.50 ± 1.00	12.33 ± 0.58	11.00 ± 1.53
Plasmodium ovale	-	14.33 ± 0.58	12.67 ± 0.58	12.33± 0.58	13.33 ± 0.58	13.00± 0.58	11.00 ± 1.00	09.33 ± 0.58
Tricho. sp.	-	15.33 ± 0.58	14.33 ± 0.58	13.67± 0.58	15.00± 0.58	14.00± 0.58	12.00 ± 1.00	11.00 ± 0.58
Micro. sp.	-	13.67 ± 0.58	12.33 ± 1.53	12.00± 1.00	13.00 ± 1.00	12.50 ± 1.33	10.50 ± 0.58	09.00 ± 1.00
Cryptococcus neoformans	-	14.67 ± 0.58	12.00 ± 2.00	12.67± 0.58	14.00 ± 2.00	12.00 ± 1.00	11.33 ± 1.00	10.00 ± 0.58

MZI: Mean zone of inhibition (mm); zone of inhibitions under 8 mm were considered as less active and were discarded. MP = Market Product, MELI = Methanol extract of *L. indica*, MELM = methanol extract of *L. macrophylla*.

S. sonnei, B. subtilis and *E. coli* respectively. But the tested extract produced no inhibition against the *P. aeruginosa* and *S. typhi* (Table 2).

The standard (Erythromycin) was active against all the tested bacteria within the range of 25.00 mm to 45.00 mm at the dose of 30 µg/disc. But among the market product MP_1 and MP_2 shows almost similar anti-bacterial activity at the range of 22.67-43.50 mm and 22.33-44.00 mm. Whereas MP_3 (range: 22.00-43.33 mm) and MP_4 (range: 21.50-42.00 mm) shows lesser activity in comparison with standard, MP_1 and MP_2. In the anti-fungal activity, it also observed that the extracts and market preparation showed significant antifungal activity against the tested fungal species when compared to fluconazole as a standard. In the anti-fungal sensitivity test, it was observed that both plant extracts were found to be active against all the pathogenic fungi. The zone of inhibition of methanol extract of *L. indica* was found within the range of 10.50-12.33 mm whereas for methanol extract of *L. macrophylla* it was 09.00-11.00 mm.

The highest zone of inhibition was produced by methanol extract of *L. indica* (12.33 mm) against *Candida albicans* at the dose of 500 µg/disc. Then followed by 12.00 mm (*Aspergillus niger* & *Trichophyton spp.*), 11.33 mm (*Cryptococcus neoformans*), 11.00 (*Blastomyces dermatitidis* & *Pityrosporum ovale*) and 10.50 mm (*Microsporum spp.*) respectively (Table 3).

Whereas the highest zone of inhibition was produced by methanol extract of *L. macrophylla* (11.00 mm) against *Candida albicans* & *Trichophyton spp.* Then followed by 10.33 mm (*Blastomyces dermatitidis*), 10.00 mm (*Aspergillus niger* & *Cryptococcus neoformans*), 09.33 mm (*Pityrosporum ovale*) and 09.00 mm (*Microsporum spp.*) respectively (Table 3).

The standard (Fluconazole) at the dose of 30µg/disc was active against all the test fungi within the range of 13.67 mm to 16.33 mm. And the four market product produced almost similar activity in comparison with standard. But the MP_3 (shows higher sensitivity than the other three (Table 3).

***In vitro* minimum inhibitory concentration (MIC) test**

In general, the microorganisms showed the susceptibility to methanol extracts of *L. indica* and *L. macrophylla* with minimum inhibitory concentrations ranging from 64~512 µg/ml (Table 4). Regarding the study of MIC for the test bacteria, *B. cereus* and *B. megateriuum* showed the susceptibility to methanol extracts of *L. indica* with MIC at 512 µg/ml and for *B. subtilis* and *P. aeruginosa* concentration was at 256 µg/ml. MIC of 64 µg/ml was found for *V. cholera* and *S. dysenteriae* but no activity found for *E. coli* and *S. typhi*. There were five bacteria (*B. subtilis, V. cholera, E. coli, S. sonnei and B. megateriuum*) which showed susceptibility to methanol extracts of *L. macrophylla* at highest concentration at 512 µg/ml. No MIC was found for *S. typhi* and *P. aeruginosa within the tested concentration range.* According to the MIC test for the fungi, *Microsporum spp.* showed the susceptibility to methanol extracts of *L. indica* with MIC at 512 µg/ml and for *C. neoformans*, *P. ovale* and *B. dermatitides* the concentration was at 128 µg/ml. Lowest MIC of 64 µg/ml was found for *Trichophyton spp.*, *C. albicans*, *Aspergillus niger.* On the other hand, *Microsporum spp.* and *Pityrosporum ovale* showed the susceptibility to methanol extracts of *L. macrophylla* with MIC of 512 µg/ml and for *A. niger*, *B. dermatitides* and *C. neoformans* the concentration was at 256 µg/ml. Lowest minimum of concentration at 128 µg/ml found for *Trichophyton spp.* and *C. albicans*. It is known that a broad range of secondary metabolites found in the plants that consists of tannins, terpenoids, alkaloids and flavonoids has antimicrobial potential [39]. So, the extracts might contain such types of phytochemicals which is responsible for the antimicrobial activities found in present study (Table 5).

CONCLUSION

The present pharmacological study and comparative evaluation of methanol extracts of *L. indica and L. macrophylla* indicates that the plant contains effective bioactive compounds that can be used for the treatment of diarrheal and microbial diseases and can be a potential source of biologically important drug candidates. However, further research is necessary to find the different lead compounds, isolate them in pure form and determine their full spectrum of efficacy, their safety and feasibility of use of them on human subjects.

Table 4: Minimum inhibitory concentration for the test bacteria by the treatment groups

Test organisms	Minimum inhibitory concentrations (µg/ml)		
	MELI	MELM	Erythromycin
Bacteria			
Bacillus subtilis	256	512	2
Bacillus megateriuum	512	512	2
Bacillus cereus	512	128	2
Staphylococcus aureus	128	256	< 1
Escherichia coli	Nil	512	2
Pseudomonas aeruginosa	256	Nil	4
Shigella dysenteriae	64	128	4
Shigella sonnei	128	512	4
Salmonella typhi	Nil	Nil	4
Vibrio cholera	64	512	4

Table 5: Minimum inhibitory concentration for the test fungi by the treatment groups

Test organisms	Minimum inhibitory concentrations (µg/ml)		
	MELI	MELM	Fluconazole
Fungi			
Aspergillus niger	64	256	1
Blastomyces dermatitides	128	256	1
Candida albicans	64	128	2
Pityrosporum ovale	128	512	2
Trichophyton spp.	64	128	2
Microsporum spp.	256	512	2
Cryptococcus neoformans	128	256	2

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

The authors greatly acknowledge to respective authority of the Department of Pharmacy, Southern University Bangladesh (SUB), for providing valuable laboratory facilities.

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