

PHARMACOLOGICAL INVESTIGATIONS OF *LITSEA LANCIFOLIA* (ROXB.) HOOK. F.

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Abstract

The petroleum ether, chloroform and ethyl acetate soluble fractions of methanolic extract of *Litsea lancifolia* Roxb. leaves were subjected to different pharmacological screenings to explore its potential as anti-oxidant, antimicrobial, peripheral analgesic, hypoglycemic and CNS depressing agent. The ethyl acetate soluble fraction showed highest total phenolic content and free radical scavenging activity compared to the standard, acetyl salicylic acid. Potential antimicrobial activity was shown against *P. aeruginosa* (23.50 mm), *E. coli* (22.33 mm), *B. cereus* (18 mm) and *S. paratyphi* (18 mm). The crude extract demonstrated significant peripheral analgesic ($p < 0.01$) activity with 69.45 and 77.96% inhibition of acetic acid-induced writhing at 100 and 200 mg/kg b.w., respectively. The crude methanolic extract also showed significant hypoglycemic activity ($p < 0.01$) at a dose of 500 mg/kg/day on the 7th day of treatment. All the organic soluble fractions exhibited noteworthy ($p < 0.001$) CNS depressant activity. Taken together, the plant can be considered as a good material for further chemical investigation to isolate the bioactive constituents.

Bangladesh is a great source of medicinal plants and for the purposes of traditional medication about 500 species are being used here (Yusuf *et al.* 1994). Plants remain as the novel source of structurally important compounds but a few of them have been validated by scientific criteria and sometimes-medical research does not support their effectiveness by experimental studies. *L. lancifolia*, which is used as alternative medicine by the people of Chittagong Hill Tracts, Bangladesh, was selected to study its biological activities, as there are very few reports for its biological studies. *Litsea lancifolia* (Roxb.) Hook. f. which is named as Judijaylla by Chakma tribes belonging to the family Lauraceae is distributed in the forests of southeastern part of Bangladesh. Warm root extract is used frequently by Chakmas to cure diarrhea in Rangamati (Yusuf *et al.* 2009). A new bis-benzylisoquinoline, lancifoliaine and seven known alkaloids, namely *N*-allyllauroitsine, reticuline, actinodaphnine, norboldine, pallidine, cassythicine and boldine were isolated from the stem bark of *L. lancifolia* (Sulaiman *et al.* 2011).

As part of on going investigation on medicinal plants of Bangladesh (Bulbul *et al.* 2016, 2017), the crude methanol extract of leaves and its petroleum ether, chloroform and ethyl acetate soluble fractions of *L. lancifolia* were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging, antimicrobial, analgesic, hypoglycemic and CNS depressant activities for the first time and the results of the preliminary investigations are reported.

The plant sample of *Litsea lancifolia* (DACB-35164) was collected from Chittagong Hill Tract, Bangladesh and was identified by Bangladesh National Herbarium, Dhaka for future reference. The collected plant materials were cleaned, sun-dried and pulverized. The grounded plant material (800 g) was soaked in methanol at room temperature for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman No. 1 filter paper. The filtrates were concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (10 g) of the concentrated methanol extract was fractionated by using the protocol designed by Kupchan

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and Tsou (1973) and modified version of van Wagenen *et al.* (1993). In brief, 5 g of the crude extract was triturated with 90% methanol in water. The prepared solution was then fractionated successfully using solvents of increasing polarity, such as petroleum ether, chloroform and ethyl acetate. All the organic soluble fractions were evaporated to dryness by using rotary evaporator at low temperature and kept in airtight containers for further analysis.

Table 1. Kupchan partitionates of *Litsea lancifolia* leaf.

Crude extract/fractions*	Leaf (g)
ME	5.00
PESL	0.87
CSF	0.50
EASF	0.45

*ME = Methanolic crude extract; PESF= Petroleum ether soluble fraction, CSF = Chloroform soluble fraction and EASF= Ethyl acetate soluble fraction.

Gallic acid, Folin-Ciocalteu reagent, DPPH, ascorbic acid, kanamycin, indomethacin, alloxan, metformin hydrochloride, diazepam, acetic acid, tween-80 and 0.9% NaCl saline solution were used in the investigation. The extracts of the plant were dissolved in 1% Tween-80 and then in 0.9% normal saline separately at a concentration of 10 mg/ml and the required dose was administered according to the weight of each mouse.

Swiss-albino mice of either sex aged 4 - 5 weeks, average weight 20 - 25 g were used for the experiment. All the procedures in this study for animal handling were performed according to the protocol of Animal Resources Branch of ICDDR,B. All efforts were made to minimize animal sufferings and to reduce the number of animals used in the experiments. They were kept in standard environmental condition (at $24.0 \pm 1^\circ\text{C}$ and 55 - 65% relative humidity and 12 hrs light/12 hrs dark cycle) for a week for acclimation after their purchase and fed with rodent food purchased from ICDDR,B and water *ad libitum*.

The total phenolic content of the extracts was determined with Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd (2006). Following the method developed by Brand-Williams *et al.* (1995), the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, ascorbic acid as reference standard. Antimicrobial activity was determined by the disc diffusion method (Bauer *et al.* 1966). Peripheral analgesic activity was evaluated by formalin-induced paw licking method (Pourmotabbed *et al.* 2011).

Hypoglycemic activity was evaluated by Sekar *et al.* (1990) in the present experiment, 20 mice (16 diabetic, 4 normal mice) were used. The mice were divided into five groups each containing four mice as shown below:

- Group I: Normal control (Non diabetic) mice were administrated 1 ml of distilled water
- Group II: Diabetic control (Untreated group) mice were untreated.
- Group III: Diabetic mice were administrated metformin (100 mg/kg b.w.) daily using an intragastric tube for 7 days.
- Group IV: Diabetic mice were administrated methanolic extract of *L. lancifolia* (300 mg/kg b.w.) daily for 7 days.
- Group V: Diabetic mice were administrated methanolic extract of *L. lancifolia* (500 mg/kg b.w.) daily for 7 days.

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean \pm SD.

The petroleum ether, chloroform and ethyl acetate soluble fractions of the crude methanol extract as well as Kupchan partitionates of *L. lancifolia* were evaluated for the antioxidant, antimicrobial, peripheral analgesic, hypoglycemic and CNS depressant activities bioassay.

The total phenolic content of the extractives of leaves of *L. lancifolia* was found to range from 15.96 ± 0.23 to 79.93 ± 0.63 mg of GAE/g of extractives, with the highest amount of phenolics 79.93 ± 0.63 being observed in the ethyl acetate soluble fraction (Table 2). In the DPPH free radical scavenging assay, the chloroform fraction of leaves of *L. lancifolia* showed maximum free radical scavenging activity having IC_{50} value of 64.01 ± 0.30 $\mu\text{g/ml}$ while the standard ascorbic acid showed IC_{50} value of 51.54 ± 0.17 $\mu\text{g/ml}$ (Table 2).

Table 2. Total phenolic content and DPPH free radical scavenging activity of *L. lancifolia*.

Plant sample/ standard	Total phenolic content (mg of GAE/gm of extract)	DPPH free radical scavenging activity (IC_{50} $\mu\text{g/ml}$)
PESF	15.96 ± 0.23	65.95 ± 0.69
CSF	33.35 ± 0.55	64.01 ± 0.30
EASF	79.93 ± 0.63	75.44 ± 4.80
Ascorbic acid		51.54 ± 0.17

PESF = Petroleum ether soluble fraction, CSF = Chloroform soluble fraction and EASF = Ethyl acetate soluble fraction.

Table 3. Zones of growth inhibition (mm) showing antimicrobial activity for three fractions of *L. lancifolia*.

Microorganisms	PESF	CSF	EASF	Kanamycin/ griseofulvin
<i>Bacillus subtilis</i>	11.00 ± 0.82	17.33 ± 0.47	14.67 ± 0.47	35.00 ± 0.82
<i>B. megaterium</i>	13.33 ± 0.47	16.50 ± 0.41	8.67 ± 0.47	33.33 ± 0.47
<i>B. cereus</i>	8.17 ± 0.62	18.33 ± 0.47	14.17 ± 0.24	35.33 ± 1.25
<i>Escherichia coli</i>	11.75 ± 0.75	12.83 ± 0.85	22.33 ± 0.47	33.00 ± 0.82
<i>Pseudomonas aeruginosa</i>	23.50 ± 0.50	9.33 ± 0.47	-	30.67 ± 0.47
<i>Salmonella paratyphi</i>	-	18.50 ± 0.41	8.83 ± 0.85	32.17 ± 0.24
<i>Sarcina lutea</i>	-	10.67 ± 0.94	8.50 ± 0.41	33.50 ± 0.41
<i>Shigella dysenteriae</i>	17.50 ± 1.50	14.33 ± 0.94	-	33.67 ± 0.47
<i>S. boydii</i>	13.50 ± 0.50	18.17 ± 0.24	-	32.67 ± 0.47
<i>Staphylococcus aureus</i>	18.33 ± 1.25	14.67 ± 0.47	10.17 ± 0.24	33.33 ± 0.47
<i>Vibrio mimicus</i>	14.75 ± 0.25	-	10.67 ± 0.94	31.83 ± 0.24
<i>V. parahemolyticus</i>	-	9.33 ± 0.47	11.67 ± 0.47	32.50 ± 0.41
<i>Candida albicans</i>	-	12.67 ± 0.94	9.50 ± 0.41	30.67 ± 0.47
<i>Aspergillus niger</i>	-	9.17 ± 0.62	10.67 ± 0.47	31.67 ± 0.47
<i>Sacharomyces cereveceae</i>	-	7.33 ± 0.47	-	30.50 ± 0.41

Values for zone of growth inhibition are presented as mean \pm Sd. - Indicates inhibition (disc diameter is 5.0 mm)

The result of the investigation for the antibacterial activity of different partitionates of *L. lancifolia* has been summarized in Table 3. The Petroleum ether soluble fraction (PESF) of *L. lancifolia* showed potent antimicrobial activity against *P. aeruginosa* (23.50 mm), very good activity against *S. aureus* (18 mm) and *S. dysenteriae* (17.50 mm), whereas exhibited mild to

moderate activity against *B. subtilis* (11 mm), *B. megaterium* (13 mm), *E. coli* (11 mm) and *V. mimicus* (14 mm). The chloroform soluble fraction (CSF) revealed very good activity against *B. cereus*, *S. paratyphi*, *S. boydii* (18 mm), *B. subtilis* (17 mm) and *B. megaterium* (16.50 mm). The CSF showed mild to moderate activity (9 to 14 mm) against *S. aureus*, *S. lutea*, *E. coli*, *P. aeruginosa*, *V. parahemolyticus*, *S. dysenteriae*, *C. albicans*, *A. niger* and *S. cereveceae*. The ethyl acetate soluble fraction (EASF) demonstrated potent antimicrobial activity against *E. coli* (22.33 mm) whereas moderate activity against *B. subtilis* (14.67 mm) and *B. cereus* (14.17 mm).

The leaf extract of *L. lancifolia* showed statistically significant peripheral analgesic activity at both doses of 100 and 200 mg/kg body weight with writhing inhibition of 69.45 and 77.96%, respectively (Table 4).

Table 4. Peripheral analgesic activity of methanolic crude extract of *L. lancifolia*.

Group	Drug/ Test sample (Dose)	No. of writhing	% of inhibition
Group I (Control)	Saline water, Tween 80	14.75 ± 3.21	-
Group II (Standard)	Indomethacin, 10 mg/kg b.w.	3.00 ± 1.20***	79.66
Group III	<i>L. lancifolia</i> 100 mg/kg b.w.	4.50 ± 3.21**	69.45
Group IV	<i>L. lancifolia</i> 200 mg/kg b.w.	3.25 ± 2.075**	77.96

All values are expressed as mean ± SEM, (n = 6); One way ANOVA followed by DMRT. ***p < 0.001, **p < 0.01, *p < 0.05, significant compared to control.

Table 5. Hypoglycemic Activity of methanolic crude extract of *L. lancifolia*.

Groups	Blood glucose level			
	1st day	3rd day	5th day	7th day
Control (non-diabetic)	5.20 ± 0.17	5.01 ± 0.13	5.50 ± 0.35	4.87 ± 0.26
Control (diabetic)	10.65 ± 0.22	10.16 ± 0.49	12.54 ± 0.32	11.93 ± 0.51
STD (Metformin HCl) 50 mg/kg b.w./day	12.46 ± 0.67	5.53 ± 0.27**	4.46 ± 0.14**	4.26 ± 0.32**
MELL 300 mg/kg b.w./day	12.85 ± 1.63	10.8 ± 1.2	8.15 ± 0.77	7.6 ± 0.44
MELL 500 mg/kg b.w./day	11.5 ± 0.63	9.3 ± 0.94	7.7 ± 0.54	7.0 ± 0.75*

All values are expressed as mean ± SEM, (n = 6); ANOVA followed by DMRT. ***p < 0.001, **p < 0.01, *p < 0.05 significant compared to control.

Table 6. CNS depressant activity of methanol extract of *L. lancifolia* leaves.

Treatment	Doses	Number of movements				
		0 min	30 min	60 min	90 min	120 min
1% Tween 80 in Saline water (Control)	10 ml/kg b.w./day	13.50 ± 1.19	14.00 ± 1.29	14.25 ± 0.85	14.00 ± 1.08	13.50 ± 0.29
Diazepam (Standard)	1 mg/kg b.w./day	10.75 ± 0.48	6.75 ± 0.25*	4.00 ± 0.48*	2.75 ± 0.48	1.50 ± 0.29*
PESF	500 mg/kg b.w./day	5.75 ± 0.48***	3.75 ± 0.48***	3.00 ± 0.41***	1.75 ± 0.48***	1.75 ± 0.25***
CSF	500 mg/kg b.w./day	6.50 ± .65***	0.50 ± 0.41***	3.50 ± 0.29***	2.00 ± 0.41***	1.50 ± 0.29***
EASF	500 mg/kg b.w./day	6.00 ± 0.41***	4.00 ± 0.58***	3.50 ± 0.29***	1.750 ± 0.48***	1.75 ± 0.25***

All values are expressed as mean ± SEM, (n = 6); ANOVA followed by DMRT. ***p < 0.001, **p < 0.01, *p < 0.05 significant compared to control.

The effects of methanol extract of *L. lancifolia* leaves on blood glucose level in alloxan induced diabetic rats are shown in Table 5 which indicated that the blood glucose level significantly decreased ($p < 0.05$) on 7th day of treatment after administration of 500 mg/kg/day of the extract.

In this test, the extract showed a decrease in locomotion in the test animals. The number of crossing hole from one chamber to another by mice of the control group remained almost steady from 0 to 120 min (Table 6). But the three different fractionates at 500 mg/kg dose showed significant and gradual decrease of movement from 0 to 120 min. This result of the study showed to have CNS depressant potential in *L. lancifolia*.

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