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Pharmacological and Phytochemical Screening of Ethanol Extract of *Litsea monopetala* (Roxb.) Pers.

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ABSTRACT

Present study was conducted by the ethanol extract of leaves of *Litsea monopetala*. Preliminary phytochemical screening with the crude extract demonstrated the presence of alkaloids, tannins, steroids, flavonoids and reducing sugars. *In-vitro* antimicrobial sensitivity was evaluated against 4 Gram positive and 7 Gram negative pathogenic bacteria and 7 fungi using ciprofloxacin and fluconazole respectively as standards. In disc diffusion antimicrobial assay, *L. monopetala* showed varying degrees of antimicrobial activities with zone of inhibition ranging from 10-12 mm and 12-14 mm for test bacteria and fungi respectively where the growth of *Pityrosporum ovale* and *Cryptococcus neoformans* were strongly inhibited. In the minimum inhibitory concentration (MIC) test by serial dilution method, a mild to strong MICs were observed for the test microorganisms. During the *in-vitro* clot lysis test, the crude extract exhibited percent lysis of clot significantly by 18.84% compared to the standard, SK (81.53%). Moreover, the extract produced inhibition of protein denaturation and haemolysis by 40% and 42.44% in the *in-vitro* anti-inflammatory and membrane stabilization test.

Keywords: *Litsea monopetala*, antimicrobial, MIC, anti-inflammatory, membrane stabilization, anti-atherothrombosis.

INTRODUCTION

There is a growing focus on the importance of medicinal plants and traditional health systems in solving the health care problems of the world. Because of this awareness, the international trade in plants of medical importance is growing phenomenally, often to the detriment of natural habitats and other populations in the countries of origin. Most developing countries have viewed traditional medical practice as an integral part of their culture. In spite of this traditional health care systems suffered a setback during colonial times and lost patronage particularly in urban areas.[1] *Litsea monopetala* (Roxb.) Pers. (Family: Lauraceae; Bengali: Bara-kukurchita); is a small tree up to 18 m tall, with a straight to crooked trunk, up to 60 cm in diameter.[2] The bark is mildly astringent, stomachic and stimulant; after being bruised, applied to contusions. Water extract of the bark is given with sugar to treat diarrhoea and dysentery. Powder of the bark is applied to body for pains arising from blows or bruises or from hard work; it is also applied to fracture in animals.[3]

The main objective of the study was to detect different chemical groups of the ethanol extract of *L. monopetala* and to evaluate some pharmacological activities.

MATERIALS AND METHODS

Collection and identification

For the investigation, the leaves of the *L. monopetala* were collected from the "Botanical Garden and Eco Park" Sitakundo, Chittagong, Bangladesh and was identified by Forest Research Institute (FRI); Chittagong, Bangladesh.

Extraction

After collection, the plant was spattered from other plant parts and dust and then washed with running tap water. The plant was then subjected for shade dry at temperature not exceeding 50ºC. Then the leaves were ground into coarse powder and about 150 gm of powdered material was subjected for hot extraction with 750 ml of ethanol (99.8%) (Soxhlet apparatus; Quickfit, England). Extraction was carried out for 18 h and then it was made to concentrate at room temperature. Yield of the crude extract was 8.96%.

Preliminary screening for phytoconstituents

For preliminary phytochemical screening, the crude ethanol extract was subjected to various tests (Table-1) for determination of chemical nature of the extract.

Antimicrobial screening

The antibacterial and antifungal activities of the crude extract were evaluated by the disc diffusion method[4] against 4 Gram positive (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus* and *Staphylococcus aureus*) and 7 Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi*, *Vibrio cholerae* and *Salmonella paratyphi*) pathogenic bacteria and 7 pathogenic fungi (*Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton longifusis*, *Microsporum canis* and *Cryptococcus neoformans*) using ciprofloxacin and fluconazole as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was determined by measuring the diameter of zones of inhibition expressed in mm. The experiments were carried out in triplicate and the results have been shown as mean ± SD (standard deviation).

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of ethanol extract of *L. monopetala* was determined by the serial tube dilution technique[5] in nutrient broth medium (Hi Media Laboratories Ltd., India), containing graded concentration of the plant extract inoculated with the test organisms.

Anti-inflammatory activity test

Table 3 shows the anti-inflammatory effect of the crude ethanol extract of the subjected plant. To conduct the experiment, 3 clean centrifuge tubes were taken one for standard, one for control and the rest for crude extract. The tubes were marked accordingly. 1 ml of 5% egg albumin solution was kept into all tubes; 1 ml of ethanol was added to the control tubes. 1 ml acetyl salicylic acid (0.1 mg) (ASA; Reckitt Benckiser Ltd., Bangladesh) was mixed for positive control group. On the other hand, for test group 1 ml of (250 and 500 mg/kg) ethanol extract was mixed to the test marked tubes. The pH (5.6±0.2) of the reaction mixtures was adjusted by 1N HCl. All the reaction mixtures were wormed at 57°C for 20 min. After cooling and filtering (Whatmann filter paper no. 1), the absorbance was measured spectrophotometrically at 660 nm. The test was repeated for three times.[6]

Membrane stabilization activity test

Table 4 shows the membrane stabilization effect of the ethanol extract of the plant. For this study, 4 clean centrifuge tubes were taken. One for standard, one for control and the rest one for the crude ethanol extract. The tubes were marked accordingly. 1 ml of 10% RBCs suspension were kept into all treatment tubes, 1 ml ethanol and 1 ml acetyl salicylic acid were added to the control and positive control tubes respectively. On the other hand for test group 1 ml of (250 and 500 mg/kg) ethanol extract was mixed to the test tubes as marked. All the tubes were then treated with 1 ml hypotonic solution (0.5% NaCl solution). The pH (7.4±0.2) of the reaction mixtures was adjusted by phosphate buffer (pH 10). All the centrifuge tubes containing reaction mixtures were wormed in a water bath at 56°C for 30 min. At the end of the warmth, the tubes were cooled under running tap water. The reaction mixtures were centrifuged at 2500 rpm for 5 min. After cooling and filtration, the absorbance of the supernatants was taken at 556 nm. The test was repeated for three times.[6]

Anti- atherothrombosis activity

The thrombolytic activity of the extract was evaluated by the method developed by Daginawala et al., 2006 using streptokinase (SK) as standard drug. Dried 100 mg extract of the plant was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22-micron syringe filter. For clot lysis venous blood drawn from healthy volunteers was distributed in different pre-weighed sterile alpin tubes (1 ml/tube) and incubated at 37°C for 45 min. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each reaction tube containing pre-weighed clot, 100 µl aqueous solution of crude extract was added. Then, 100 µl of streptokinase (SK) and 100 µl of distilled water were separately added to the positive and negative controls respectively. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below: -

$$\% \text{ of clot lysis} = (\text{wt of released clot} / \text{clot wt}) \times 100$$

Statistical Analysis

All the tests were performed for three times and values are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Preliminary screening for plant's secondary metabolites with the crude ethanol extract demonstrated the presence of alkaloids, tannins, steroids and reducing sugars (Table 1).

In the disc diffusion anti-bacterial activity test, the zones of inhibition were found within the range of 10-12 mm. The highest zone of inhibition (12 mm) was obtained against *S. aureus* and *V. cholera* then followed by 11, 11, 11, 10 and 10 mm against *B. cereus*, *P. aeruginosa*, *S. paratyphi*, *E. coli* and *S. sonnei* respectively. But the extract was inactive against *S. typhi*. Ciprofloxacin was taken as standard.

During the anti-fungal test, the zones of inhibition were found within the range of 12-14 mm. The highest zone of inhibition (14 mm) was obtained against *P. ovale*. Then followed by 13, 13, 12 and 12 against *T. longifusis*, *C. neoformans*, *C. albicans* and *M. canis* respectively. The crude extract strongly inhibited the growth of *P. ovale* and *C. neoformans* in comparison to the standard fluconazole (Table 2).

Crude extract of *L. monopetala* inhibited the growth of *V. cholera*, *P. ovale* and *T. longifusis* significantly at the dose of 31.25 µg/ml then followed by *S. aureus* by 62.50 µg/ml; *P. aeruginosa* and *C. albicans* by 125 µg/ml and *B. cereus*, *E. coli*, *S. sonnei*, *M. canis* and *C. neoformans* by 250 µg/ml. But it could not act effectively against the other tested species (Table 2).

In the *in-vitro* anti-inflammatory test, the crude ethanol extract at the doses of 250 and 500 mg/kg produced mean inhibition of protein denaturation by 23% and 40% ($p<0.05$) whereas for ASA it was found to be 52% (Table 3).

Test extract 250 mg/kg and 500 mg/kg inhibited the heat induced total haemolysis of RBCs by 27% and 42.44% respectively whereas, the standard; ASA showed 89.83% of inhibition of heat induced haemolysis (Table 4). The membrane stabilization activity for crude ethanol extract of *L. monopetala* was found to be mild.

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 min of incubation at 37°C, showed 81.53% clot lysis. Clots when treated with 100 µl water (negative control) showed only negligible clot lysis (2.49%). 18.84% lysis of clot was obtained after treatment of clots with 100 µl of plant extract (Table 5).

Incase of anti-inflammatory, membrane stabilization and clotlysis activity tests, dose response phenomena were observed.

CONCLUSION

Ethanol extract of *Litsea monopetala* subjected for chemical and biological investigations demonstrated the presence of some important secondary plant materials as well as significant antimicrobial, anti-inflammatory and anti-atherothrombosis activities. But the responsible chemical moieties and their possible mechanisms are still to be found out. So, the results obtained from this study indicate that this plant species could be useful in the search for new natural bioactive compounds.

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Table 1: Phyto-constituents of the ethanol extract of *L. monopetala*

Phytochemicals	*Alkaloids*	*Glycosides*	*Tannins*	*Steroids*	*Flavonoids*	*Saponins*	*Redicing sugars*
Consequences	+++	-	++	++	+++	-	+++

(+): Presence; (-): Absence

Table 2: Antimicrobial activity of crude extract and standards

Test microorganisms	Zone of inhibition (MZI±SD) mm		MICs (µg/ml)
	EELM (500 µg/disc)	Standard (30 µg/disc)	
Gram positive bacteria		**Ciprofloxacin**	
B. cereus	11±1.00[b]	13 ±1.26	250
S. aureus	12±1.73[b]	14±0.76	62.5
Gram negative bacteria			
P. aeruginosa	11±1.73[b]	11±1.04	125
E. coli	10±1.00[a]	15±0.50	250
S. sonnei	10±1.00[a]	14±0.29	250
V. cholerae	12±1.00[b]	14±0.29	31.25
S. paratyphi	11±1.00[b]	12±1.50	Nd
Fungi		**Fluconazole**	
C. albicans	12±1.00[b]	13±0.50	125
P. ovale	14±1.00[a]	13±1.26	31.25
T. longifusis	13±1.00[a]	14±0.50	31.25
M. canis	12±1.00[b]	12±1.32	250
C. neoformans	13±1.00[b]	12±1.50	250

[a]p<0.02, [b]p<0.10
MZI: Mean zone of inhibition (mm); Zone of inhibitions under 9 mm were considered as less active and were discarded; Nd: Not determined; MICs: Minimum inhibitory concentrations; EELM: Ethanol extract of *L. monopetata.*

Table 3: Anti-inflammatory test tabulation for crude extract and controls

Controls/drugs	Inhibition of protein denaturation
Control (ethanol)	1.09±0.05
Positive control (ASA 0.1 mg)	52±0.0009[a]
EELM (250 mg/kg)	23.0±0.07[b]
EELM (500 mg/kg)	40.0±0.005[b]

[a]p<0.001, [b]p<0.05; ASA: Acetyl salicylic acid

Table 4: Tabulation for *in-vitro* membrane stabilization test

Test groups	Inhibition of haemolysis
Control (ethanol)	1.51±0.07
Positive control (ASA, 0.1 mg)	89.83±0.004[a]
EELM (250 mg/kg)	27.0±0.004[a]
EELM (500 mg/kg)	42.44±0.007[b]

[a]p<0.01,[b]p<0.10

Table 5: Anti-atherothrombosis activity of *L. monopetala* and controls

Controls/extract	Clot lysis (%)
DW (Negative control)	2.49±0.55
SK (Positive control)	81.53±5.37[a]
EELM	18.84±2.52[a]

[a]p< 0.001; DW: Distilled water; SK: Streptokinase

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