- Short communication

Antioxidant and Cytotoxic Activitiy of *Limonia acidissima* L.

Sadia Shermin, Fahima Aktar, Monira Ahsan and Choudhury M. Hasan

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

The crude methanol extract of the stem bark of *Limonia acidissima* L. and its different organic soluble partitionates were screened for cytotoxic and antioxidant activities. The brine shrimp lethality bioassay was used to evaluate the cytotoxicity. The petroleum ether soluble fraction (PESF) of the methanolic extract exhibited strong cytotoxic activity (LC_{50} = 2.0779 µg/ml). On the other hands chloroform soluble fraction of the methanolic extract showed the highest free radical scavenging activity with IC_{50} value 18.80 µg/ml.

The plant *L. acidissima* (Family- Rutaceae) is known as Kath bael in Bangla and is a common plant of Bangladesh. Its leaves, bark and then fruits have medicinal values and used as traditional medicines for centuries due to their antimicrobial,[1] antifungal,[2,3] astringent, anti-inflammatory[4] and insulin secretogouge[5] activities. In the south Asian countries like Mayanmar, Thailand etc., the bark of the tree is used as a popular natural cosmetic.[6] Different parts of the plant are also used as insecticides, anti-rodent in animal husbandry.[7-9]

Previous phytochemical investigations led to the isolation of coumarins, phytostrols, tyramine derivatives, phenolic acids, limonoids, flavonones etc from *L. acidissima.*[10-18] Since this plant has various medicinal properties so the present study was undertaken to evaluate the antioxidant and cytotoxic activities of methanolic extract of bark of *L. acidissima* systematically.

Correspondence to: Choudhury Mahmood Hasan
Contact no.: +8801819253698
E-mail: cmhasan@gmail.com

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Plant sample (stem bark) of *L. acidissima* was collected from Pabna in November, 2008. A voucher specimen (DACB - 35734) for this collection has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka-1216. The samples were cut into small pieces and sun dried for 7 days followed by oven drying for 24 hours at 40°C. The powdered material (300 g) was then soaked in 1.50 liter of methanol in a large conical flask for 7 days with occasional shaking and stirring. The whole mixture was filtered off through a cotton plug followed by Whatman filter paper no.1 and the filtrate was concentrated using a Buchi rotary evaporator. The crude extract was then fractioned by using the modified Kupchan partitioning protocol.[19]

DMSO solutions of all the extractives were applied against *Artemia salina* in a one-day *in vitro* assay.[20] For the experiment, 4 mg of each of the fractions was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/ml were obtained by serial dilution technique. Vincristine sulphate and DMSO were used as the positive and negative control, respectively. Table 1 shows the results of the brine shrimp lethality bioassay after 24 hr exposure of the shrimps to the test samples.

The antioxidant (free radical scavenging) activity of the partitionates on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method developed by Brand-Williams *et al.*[21,22] Here, 2.0 mg of each of the test sample was dissolved in methanol and solution of varying concentrations such as 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/ml were obtained by serial dilution technique. Then 2 ml of each of the test

sample was mixed with 3 ml of a DPPH-methanol solution (20 µg/ml) and was allowed to stand for 20 minutes for the reaction to occur. The absorbance was determined at 517 nm and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

% inhibition = [1- (ABS_{sample} / $ABS_{control}$)] x 100

Then % inhibitions were plotted against respective concentrations used and from the graph the IC_{50} was calculated using ascorbic acid, as the positive control. Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

The present study was undertaken to evaluate the cytotoxic and antioxidant activities of the organic soluble portion of a methanol extract of *L. acidissima* and the results have been summarized in Table 1. In the brine shrimp lethality bioassay, the pet ether soluble partitionate (PE) showed strong cytotoxic activity with LC_{50} value of 2.0779 µg/ml. The chloroform soluble partitionate (CL) exhibited significant lethality having LC_{50} value of 6.8975 µg/ml, while the crude metanolic extract (ME) demonstrated moderate activity against shrimp nauplii with the LC_{50} of 20.6226 µg/ml (Table 1 & Figure 1). In case of screening for antioxidant activity (Table 1 & Figure 2), the chloroform soluble fraction (CL) of crude methanolic extract showed the highest free radical scavenging activity with IC_{50} value 18.8 µg/ml. At the same time the pet ether soluble fraction (PE) also exhibited strong antioxidant potential having IC_{50} value of 37.64 µg/ml.

Table 1. Free radical scavenging and cytotoxic activity of different partitionates of *L. acidissima*

Sample	Antioxidant activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
VS	-	0.451 ± 1.25
BHT	17.69 ± 0.89	-
ME	292.16 ± 1.07	20.62 ± 0.76
PE	37.64 ± 1.19	2.077 ± 0.42
CL	18.8 ± 0.78	6.89 ± 0.58

Where, VS = Vincristine sulphate, BHT = Butylated Hydroxy Toluene, ME = Methanolic Extract of Bark of Plant, PE= Pet Ether Soluble Fraction, CL= Chloroform Soluble Fraction.

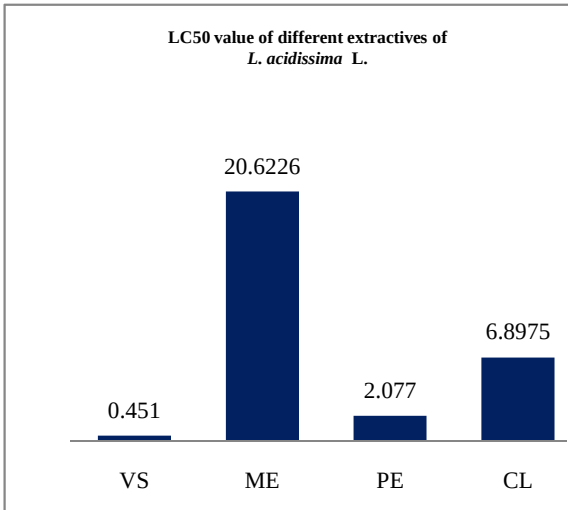

Figure 1. LC_{50} values of the different extractives of *L. acidissim*

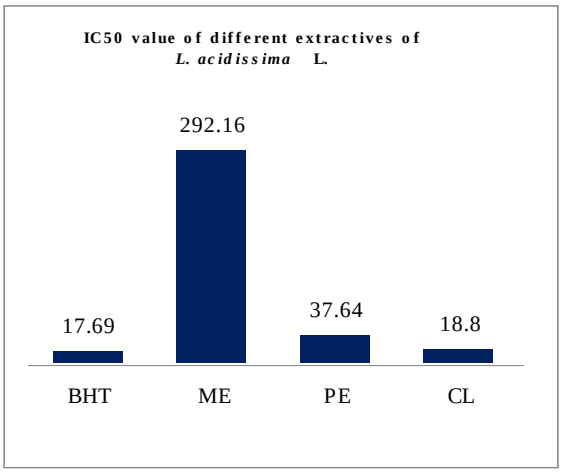

Figure 2. IC_{50} values of the different extractives of *L. acidissima*

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