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Research Article

EVALUATION OF THE ANTIOXIDANT AND CYTO-TOXIC ACTIVITIES OF THE STEM-BARK OF *LIMONIA ACIDISSIMA* (FAMILY: RUTACEAE)

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ABSTRACT

Limonia acidissima (Family: Rutaceae.) is an important medicinal plant having applications in various disorders. The aim of this study was to evaluate the anti-oxidant and cyto-toxic activities of the stem-bark of *Limonia acidissima* (Family: Rutaceae.) The stem bark of the plant *Limonia acidissima* was sun dried and extracted using methanol. Later the crude methanolic extract was fractionated into two different solvents using Petroleum ether and chloroform. The anti-oxidant activity of the different fractions was measured by the DPPH free radical scavenging activity. The chloroform soluble fractions showed very potent anti-oxidant activity by the DPPH free radical scavenging method. Evaluation of cyto-toxic activity was done using the brine-shrimp lethality bio-assay. The petroleum ether soluble fraction soluble fractions showed significant cyto-toxic potential with LC_{50} values of 0.2779 µg/ml.
Keywords: *Limonia acidissima*, anti-oxidant, DPPH, Brine shrimp.

INTRODUCTION

Plants represent a rich source of antimicrobial agent [(1)] and natural antioxidants [(2)] .Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines [(3)]. Plants generally produce many secondary metabolites which constitute an important source of anti-oxidants. Many natural substances having anti-oxidant properties have been used in health foods for medicinal and preservative purposes [(4)]. Many plant derived natural products are also used as anti-cancer agents like vincristine and vinblastine. The use of natural products to prevent cancer is becoming increasingly popular. *Limonia acidissima* (Family: Rutaceae.) is an erect, slow-growing tree with hard wood. The plant is generally 9-10 metres tall. Its Bark is rough, spiny which is ridged, fissured and scaly and there are sharp spines 0.75 to 2 inches long on some of the zigzag twigs.

Limonia acidissima is an important medicinal plant having many applications. The methanol extract of leaves showed diuretic activity in rats when given orally [(5)] .Volatile compounds obtained from the plants by the steam distillation possessed significant anti-bacterial (against *Staphylococcus aureus*), antifungal (against *Cladesporium cladesporioides*) and insecticidal properties [(6)] .Chloroform extract of the leaves exhibited antifungal activity [(7)] . *Vibrio cholerae* was

also found quite sensitive to the plant extract [8] .Chloroform extract of the stem barks showed antifungal activity [9] .Oral administration of 250 mg/kg body weight of 95% ethanolic extract of the unripe fruits of *Limonia acidissima* significantly lowered the blood glucose level of streptozotocin-induced diabetic male albino rats. Extract probably lowered the blood glucose concentration by stimulating insulin secretogouge activity [10]. Its fruit also contain acidic heteropolysaccharide which showed significant in vivo growth inhibition of Ehrlich ascites carcinoma cell [11]. Root barks contain compounds called Fernolin and Bergapten reputed to neutralize the effects of snake venoms. Protective activity against the lethal action of the venom of the jararaca (*Bothrops jararaca*) snake was confirmed by biological assays [12]

2. MATERIAL AND METHOD:

2.1 Collection of the plant sample:

Plant samples of *Limonia acidissima* L. were collected from Pabna in November, 2009 and identified by an expert taxonomist.

2.2 Preparation of plant extract:

The stem bark of the plants were separated from other plant parts and cut into small pieces. The stem barks were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried stem bark was then crushed into coarse powder by a high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka.

1.0 kg of powdered material (Stem-bark powder) was macerated with 2.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (45^0C) under reduced pressure by rotary evaporator. The obtained crude extract was 20.54 grams. 15 gm of methanolic extract was triturated with 270 ml of methanol containing 30 ml distilled water. The crude extract was dissolved completely to obtain the mother solution. This solution was partitioned successfully into two solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of Petroleum Ether was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Chloroform ($CHCl_3$) extract was collected with the help of aqueous mother fraction adding 38 ml of distilled water and keeping the other procedure unchanged. Finally Petroleum ether, Chloroform and aqueous extract were obtained.

2.3 Evaluation of antioxidant activity:

Brand-Williams [13] method was used to estimate free radical scavenging activities of the Petroleum ether and chloroform soluble fractions of the stem-bark of the plant *Limonia acidissima*. 2.0 mg of the extracts was dissolved in methanol for the experiment. Solution of different concentrations such as 500 g/ml, 250 g/ml, 125 g/ml, 62.50 g/ml, 31.25 g/ml, 15.62 g/ml, 7.8125 g/ml, 3.91 g/ml, 1.95 g/ml and 0.98 g/ml were obtained by serial dilution technique. 50 µl of methanol solution of the extract of each concentration was mixed with 5 ml of a DPPH-methanol solution (40 g/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

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

Where ABS_{sample} is the absorbance of the sample material and $ABS_{control}$ is the absorbance of the control reaction (containing all reagents except the test material). Then percent inhibitions were plotted against respective concentrations. IC_{50} values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph. Tert-butyl-1-hydroxytoluene (BHT) was used as positive control. The experiment was performed thrice and the result was expressed as mean ± Standard Error of Mean (SEM) in every case.

2.4 Evaluation of Cyto-toxic activity:

The evaluation of cyto-toxic activity was done by the Brine shrimp lethality bio-assay [14]. In this experiment simulated sea water was prepared by dissolving 38gm of sea salt in 1L of distilled water. Brine shrimp eggs were collected and hatched in a tank containing sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii.

Constant oxygen supply was carried out through the hatching time. With the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater.

Clean test tubes were taken. These test tubes were used for preparing ten different concentrations (one test tube for each concentration) of test samples. Again ten test tubes were taken for ten concentrations of standard drug Vincristine and another one test tubes for negative control test. Four mg of all the test samples (Pet ether soluble fraction, chloroform fraction) were taken and dissolved in 200µl of pure di methyl sulfoxide (DMSO) in vials to get stock solutions. Then 100µl of Solution was taken in test tube each containing 5ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100µl sample was added to test tube and fresh 100µl DMSO was added to vial. Thus ten different test tubes had different concentrations of test samples. The concentrations in ten different test tubes were 400µg/ml, 200µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml respectively. In the present study Vincristin sulphate was used as the positive control. Measured amount of the Vincristin sulphate was dissolved in DMSO to get an initial concentration of 40 g/ ml from which serial dilutions were made using DMSO to get 20 g/ml, 10 g/ml, 2.5 g/ml, 1.25 g/ml, 0.625 g/ml, 0.3125 g/ml, 0.15625 g/ml and 0.078125 g/ml. Then the positive control solutions were added to the pre marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups. For the preparation of negative control, 100 l of DMSO was added to each of three pre marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii was added to each vial. If the brine shrimps in these vials showed a rapid mortality rate, then the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration- mortality data were analyzed statistically by using Microsoft Excel program. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

3. RESULTS:

3.1. In vitro antioxidant activity:

The antioxidant activity of the different fractions of stem-bark of *Limonia acidissima* was measured on the basis of its DPPH scavenging activity. The concentration of Petroleum Ether soluble fraction and chloroform soluble fractions needed for 50% scavenging (IC_{50}) of DPPH was found to be 37.64±3.87µg/ml and 18.8±1.24µg/ml respectively. The positive control used was Butyl hydroxyl toluene (BHT) and for which the IC_{50} values were found to be 17.69±2.36µg/ml.

3.2 Cyto-toxic activity:

The pet Ether soluble fraction showed significant cyto-toxic activity with a LC_{50} value of 0.2779µg/ml. The chloroform soluble fraction showed LC_{50} value of 6.89µg/ml which was insignificant compared to the LC_{50} value of the positive control Vincristine sulfate. The LC_{50} value of Vincristine sulfate was found to be 0.445µg/ml.

4. DISCUSSION:

The current study established that the chloroform soluble fractions of the stem-bark of *Limonia acidissima* showed strong anti-oxidant activity. The anti-oxidant activities of plant extracts are mainly attributed to the presence of phenolic compounds. Therefore there is a probability that these fractions are rich in phenolic compounds. The strong cyto-toxic activities present in the Petroleum ether fractions may be due to the presence of cyto-toxic compounds. Therefore further study may be recommended to find out that the anti-cancer potential of the plant.

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