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PHYTOCHEMICAL SCREENING AND EVALUATION OF THROMBOLYTIC, MEMBRANE STABILIZING AND CYTOTOXIC ACTIVITIES OF *LAPORTEA INTERRUPTA* Linn.

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Abstract

To evaluate thrombolytic, membrane stabilizing & cytotoxic activities of *Laportea interrupta* Linn. (Family- Urticaceae) the whole plants were extracted with ethanol and were partitioned with petroleum ether, carbon tetrachloride and water for biological investigation. Different partitionates of *L. interrapta* protected the haemolysis of RBC induced by hypotonic solution and heat as compared to standard Acetyl salicylic acid. In our study ethanol extract of *L. interrupta* showed highest protection of 81 ± 0.095 by hypnotic solution and in heat 50 ± 0.496 respectively. The pet. ether soluble fraction of *L. interrupta* displayed highest thrombolytic activity (48 ± 0.207)% where Streptokinase was used as a positive control to the clots. In the brine shrimp lethality bioassay, pet. ether soluble fraction (PESF) showed significant lethality having the LC50 value 7.36 µg/ml. The obtained data provide a support for the use of our plant in traditional medicine and its further investigation.

Keywords: *Laportea interrupta,* thrombolytic activity, membrane stabilizing activity, brine shrimp lethality, traditional medicine.

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Introduction

Medicinal plants are the prime source of drugs and there are about 2000+ plant species known to possess medicinal value in the traditional Asian system of medicine[1]. The use of plant derived natural compounds which are used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. The curative properties of medicinal plants are due to the presence of various chemical substances of different composition[2]. *Laportea interrupta* commonly known as Bichhuati (odiya) or wood nettle is a plant belonging to the family Urticaceae. The leaf extract is used in fevers, rheumatic pain and headache [3]. The plant is distributed in India from sea level to high hills. It is a prostrate herb with medicinal values [4]. *Laportea* is an annual herbaceous weed around waste places. The stem is erect, angular, covered with pilose hair or stinging hairs and green in colour [5]. In West Africa, the plant finds application in ethnomedicine, in the treatment and management of headache, diuretic to cure blenorrhoea and chest problems. It is also used to deliver placenta after child birth, prevent excessive menstrual bleeding. Extracts are used to treat arthritis, anemia, hayfever, kidney problems and pain [6]. Urticaceae comprise about 45 genera and 1,000 species in the world [7]. Taxonomy of the family in Taiwan has been recently revised by Shih *et al.* (1995a, 1995b) [8, 9]. In total, 21 genera and 63 species and one variety distributed in Taiwan have been recorded [10]. Since this plant has important medicinal properties, the aim of our present work was to investigate whether our selected ethanolic extracts of *L. interrupta* are suitable for thrombolytic, membrane stabilizing & cytotoxic activities or not. For our current studies, the whole plants were extracted.

Materials and Methods

Plant materials

For this present investigation the plant *L. interrupta* was collected from, Dhaka, Bangladesh in September 2015 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. Accession no is 42291.

Preparation of extract

The collected plants were shade dried for several days and then oven dried to facilitate grinding and stored in a air tight container. The dried powder materials (about 200 gm powder) were soaked in sufficient amount of 90% ethanol for about two weeks. The container with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then filter by a piece of **clean, white cotton** materials. Then the filtrate was filtered again through Whatman filter paper, total filtrate was concentrated using a rotatory evaporator to get the crude extract. The concentrated aqueous ethanol extract was partitioned by the Kupchan method and the resultant partitionates, i.e. Carbon tetra chloride, Chloroform, Petroleum ether and Aqueous soluble materials were stored & used for our current investigations.

Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with Ferric chloride and Potassium dichromate solutions, and steroids with Libermann-Burchard reagent reducing sugars with Benedict's reagent [11,12].

Thrombolytic assay

Following the method developed by Prasad *et al.* (2007) whole blood was drawn from healthy human volunteers and 1ml of blood was transferred to the previously weighed sterile Eppendorf tubes and was allowed to form clots. After clot formation, the serum was completely removed without disturbing the clot. To each Eppendorf tube containing pre-weighed clot, 100 µl aqueous solutions of different extracts along with the crude extracts were added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control Eppendorf tubes. All tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. The released fluid was removed and Eppendorf 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 [13]:

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

Membrane stabilizing activity

The membrane stabilizing activity of the extractives was determined with human erythrocytes by following the method developed by Omale *et al.* [14] The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat induced RBC haemolysis. To prepare the erythrocyte suspension, 5 ml of whole blood was withdrawn from healthy human volunteer by hypodermal syringes (containing anticoagulant 3.1% Na-citrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH7.4) through centrifugation for 10min at 3000 rpm [15,16].

Cytotoxic investigation

20 mg of each of the extractive was weighed out and dissolved in 1 ml of DMSO (di-methyl-sulfoxide) by sonicator and finally taken in the test tubes for our studies. Then 38 gm of sodium chloride (NaCl) was weighed out and was dissolve in 1L distilled water to prepare the saline solution. Brine shrimp eggs were collected from pet shops and was hatched to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. Eight doses of each of the extractive (5, 25, 50, 100, 250, 500, 1000, 2500 µg/ml) were taken for the test. Each of the preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. For this purpose about 0.5 ml extract solution (of each of the extractive) was taken in a test tube and 9.5 ml salt water was added with it to get our desire concentration and the test tubes were allowed to sonicator. The concentration of the mother solution should always be 1 mg/ml. After making the test tubes properly, 10 living shrimps were added to each of the test tube with the help of micropipette [17]. Observation was done after 24 hours and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration. Vincristin sulphate (VS) was used as positive control.

Results

Phytochemical constituents

The ethanolic extract of *L. interrupta* (whole plants) showed the presence of alkaloids, tannins, steroids, gums, glycosides, flavonoids & reducing sugar (Table 1).

Thrombolytic assay

As a part of discovery of cardio protective drugs from natural resources, the extractive of *L. interrupta* was subjected to assay for thrombolytic activity and the results are presented in Table 2. 100 µl SK was used as a positive control to the clots and subsequent incubation for 90 minutes at 37°C, showed (65±0.081)% lysis of clot. In this study, pet. ether soluble fraction of *L. interrupta* displayed highest thrombolytic activity (48 ± 0.207)%.

Membrane stabilizing activity

Different partitionates of *L. interrupta* protected the haemolysis of RBC induced by hypotonic solution and heat as compared to standard Acetyl salicylic acid. Among the different fractionates, the ethanol extract inhibited 81 ± 0.095 and 50 ± 0.496 of haemolysis of RBC induced by hypotonic solution and heat as compared 72 ± 0.115 and 40 ± 0.577 demonstrated by acetyl salicylic acid, PESF 51.49 ± 0.233 and 32 ± 0.158, CTSF 13 ± 0.588 and 6.8 ± 0.070, ASF 34.80 ± 0.008 and 23 ± 0.20 respectively- shown in Table- 3.

Cytotoxic investigation of the plant extracts

Following the procedure of Meyer, the extracts showed lethality against the brine shrimp nauplii indicating the biological activity of the compounds present in the extracts. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with increasing concentration of the extractive. The LC_{50} value of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration. Table- 4 represents the LC_{50} value of the extractive.

Discussion

Thrombolytic drugs are obtained from various sources are used for the treatment of thrombosis.These agents are used to disrupt already formed blood clots in clinical settings where ischemia may be fatal (acute myocardial infarction, pulmonary embolism, ischemic stroke, and arterial thrombosis). thrombolytic drugs are also called "fibrinolytic drugs." There are three major classes of fibrinolytic drugs: tissue plaminogen activator (tPA), streptokinase (SK) and urokinase (UK). While drugs in these three classes all have the ability to effective dissolve blood clots. The thrombolytic effects obtained from our current study suggests that the extractives showed moderate thrombolytic activity and pet. ether soluble fraction of *L. interrupta* displayed highest thrombolytic activity (48 ± 0.207)%.

Different partitionates of *L. interrupta* protected the haemolysis of RBC induced by hypotonic solution and heat as compared to standard Acetyl salicylic acid. In this study ethanol extract of *L. interrupta* showed 81 ± 0.095 by hypnotic solution and in heat 50 ± 0.496 respectively, highest protection of haemolysis of RBC membrane.

The cytotoxic activity of the extractive is presented in table 4. The degree of lethality of extract was directly proportional to the concentration of the extract i.e., at higher concentration, the mortality was higher. Highest lethal effect was observed at extract concentration of 2500 µg/ml. LC_{50} values greater than 1000 µg/ml were considered inactive (non toxic). The LC_{50} of the pet. ether extractive was found to be 7.36 µg/ml and the extract is toxic. In our study, the extract caused dose dependent lethal effect on brine shrimp, thus the extract is toxic and purified metabolite can have antitumor activity. Brine shrimp lethality test is carried out in order to reveal new anticancer compounds.

In conclusion, it could be suggested that the crude extract of dried whole plant of *L. interrupta* Linn possess thrombolytic, membrane stabilizing and cytotoxic properties. It is clearly evident from the above findings that the plant has significant thrombolytic, membrane stabilizing and cytotoxic activities. Therefore, the plant is a good candidate for further studies.

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Table 1: Results of different chemical groups of the ethanolic extract of *L. Interrapta* (whole plants).

Plant extract	alkaloids	tannins	steroids	gums	glycosides	flavonoids	saponins	reducing sugar
Ethanolic extract	+	+	+	+	+	+	–	+

(+) means positive, (-) means negative

Table 2: Thrombolytic activity (in terms of % clot lysis) of *L. Interrupta*

Sample	% of RBC lysis
EE	33 ± 0.22
PESF	48 ± 0.207
CTSF	40 ± 0.22
ASF	41± 0.126
SK	65± 0.081
Blank	3.14 ± 0.31

SK = Streptokinase (positive control), EE= Ethanol Extract, PESF= Pet Ether soluble fraction CTSF= Carbon tetrachloride soluble fraction, ASF= Aqueous soluble fraction, Blank= Water as negative control

Table 3: Percentage (%) inhibition of heat- and hypotonic solution- induced haemolysis of erythrocyte membrane by standard and different partitionates of *l. interrupta*

Sample	% inhibition of haemolysis	
	Hypotonic solution induced	Heat induced
EE	81 ± 0.095	50 ± 0.496
PESF	51.49 ± 0.233	32 ± 0.158
CTSF	13 ± 0.588	6.8 ± 0.070
ASF	34.80 ± 0.008	23 ± 0.20
ASA	72 ± 0.115	40 ± 0.577

ASA= Acetyl salicylic acid

Table 4: Brine shrimp lethality bioassay of different extractive of whole plants of *L. interrupta* Linn.

Sample	LC_{50} (µg/ml) value of the extractive
EE	12.67
PESF	7.36
CTSF	9.42
ASF	11.11
VS	0.452

VS= Vincristin Sulphate