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PHYTOCHEMICAL INVESTIGATION, CYTOTOXIC AND THROMBOLYTIC ACTIVITY OF ACETONE EXTRACTS OF *RHYNCHOTECHUM ELLIPTICUM* (GESNERIACEAE)

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Abstract

From the archaic times *Rhynchotechum ellipticum* (Gesneriaceae) used locally as alleviates coughs in children. It is available in Chittagong and Sylhet, Bangladesh. The present study was carried out to investigate phytochemical, cytotoxic and thrombolytic activity of *Rhynchotechum ellipticum* leaf and stem of acetonic extracts . The cytotoxic activity was determined by using brine shirmp lethality bioassay and thrombolytic activity was determined in accordance with clot disruption method. For cytotoxic activity, leaf and stem extracts showed LC 50 value 71.70 and 55 µg/ml; respectively. On the otherhand, the reference standard Vincristine sulphate showed LC 50 value was 2.63 µg/ml. In the thrombolytic activity of leaf and stem extracts showed clot lysis (41.98%) and (34.21%); respectively. The standard of Streptokinase showed clot lysis (68.42%). The extracts also showed moderate antibacterial activity. It can be revealed that the extract of *Rhynchotechum ellipticum* (Gesneriaceae) possess cytotoxic and thrombolytic activity. The potential of these activities may be due to the presence of most of the phytochemicals which supports previous claims and validate its uses as an expected folk medicine.

Key words: *Leaf, Stem, Streptokinase, Clot lysis, Cytotoxic activity, Vincristine sulphate*

Introduction

Medicinal plants are always very promising for the development of new drugs and to find out any plant having medicinal activity, proper scientific screening is essential [1]. Despite the massive advancement observed in modern medicine in recent years, plants still play an important role in health care [2]. *Rhynchotechum ellipticum* (Gesneriaceae) is an erect herb, 1-2 m high with thickish stems containing many compounds like β-sitosterol, ursolic acid, stigmasterol, 3hydroxy-12-oleanane-3-O-β-D-glucopyranoside, β-daucosterol, octadecanoic acid [3]. Leaves are opposite, 16.5 cm or longer, broadly elliptic or obovate acute, minutely dentate, base cuneate, whitened beneath, above tawny as well as silkily wooly. Afterwards flowers are rose-purple, in umbellate cymes in the lower axills and berry is 6 mm diameter. It is available in Chittagong and Sylhet, Bangladesh. Traditionally it is used as alleviates coughs in children [4].

Malignant growth is an uncontrolled cell division nwhich can attack, metastasize just as spread to far off locales [5]. A portion of the anticancer operators, for example, vinblastine, irinotecan, topotecan, vincristine, taxanes are originates from plants. The seeking of tenderfoot cytotoxic operators from normal—microbial, marine just as plant sources proceeds with everywhere throughout the world [6]. Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. For example, if you are interested in developing a therapeutic that targets rapidly dividing cancer cells, researchers can look for cytotoxic compounds. Alternatively, "hits" from the initial high-throughput drug screening can be screened for unwanted cytotoxic effects before investing in drug development [7]. Chemotherapy with cytotoxic drugs is the main treatment for certain types of cancer [8].

Thrombosis is the formation of an atypical mass inside the vascular system of a living animal responsible for arterial diseases linked with myocardial infarction and stroke and venous thromboembolic disorder report for substantial morbidity and mortality [9]. Thrombin formed blood clot from fibrinogen and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator and fibrinolytic drug is the drug that has capability to dissolve thrombin in acutely occluded inside the vascular system [10]. Thrombolysis suggests the usage of thrombolytic drugs, that are either that, is obtained from Streptococcus species or using recombinant biotechnology whereby tPA is actually manufactured by bacteria, resulting in a recombinant tissue plasminogen activator together with rtPA [11]. In thrombolytic therapy different type of drugs are used, including alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots [12]. The most widely used drug for thrombolytic therapy is tissue plasminogen activator, but other drugs can do a similar thing [13]. But starting from first generation thrombolytic agents like streptokinase, urokinase to latest third generation drugs such as reteplase have side effects including internal bleeding, bronchospasm, hypertension, hemorrhagic cerebrovascular diseases etc [14]. Moreover, treatment with SK is restricted due to immunogenicity and the development of improved recombinant variants of these drugs is disturbing because of the unavailability of thrombolytic drugs [15]. The new studies and investigations in this area will give new intimate that encourage the advancement of the ideal thrombolytic treatment [16].

Therefore, the aim of the present study is to investigate the thrombolytic activity of acetonic extracts of *Rhynchotechum ellipticum* that might explore the remedial potential of this plant to a great extent.

Methods

Chemicals and reagents

Ascorbic acid, Vincristine sulphate, DMSO (Dimethyl sulfoxide), DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) were used.

Plant Materials

Rhynchotechum ellipticum(Gesneriaceae) leafs and stems were collected from Sylhet, Bangladesh

and identified by experts at Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (DACB 47042) has been submitted there for future reference.

Preparation of acetone extract

At first, a clean flat flat-bottomed glass container was taken and added about 300 gm of powdered leaves and 290 mg stems were taken in separate clean, flat-bottomed glass container. Then 1800 ml of acetone added into the container and soaked the powder into the methanol. Afterwards, the container was sealed with its contents and kept for a period of 14 days accompanying occasional shaking and stirring. After that, the coarse part of the leaf and stem were separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, it was filtered through whatman filter paper. Then the filtrate was kept in Rotary evaporator machine which separates solvent and desirable crude extract was obtained.

Phytochemical screening

Phytochemical screening of *Rhynchotechum ellipticum* was carried out to identify the functional groups asdescribed [17,18,19].

Cytotoxic activity

For cytotoxicity screening, DMSO (Dimethyl sulfoxide) solutions of the acetonic of leaf and stem extractswere applied to Artemia salina in a one-day in vivo assay. For the experiment, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (640, 320, 160, 80, 40, 20, 10, 5 µg/ml) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii was calculated for each concentration. The lethal concentration (LC50 and LC90) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration [20].

Thrombolytic activity

Preparation of extract dose: Extract Concentration, Stock solution = 100mg/10ml. Standard: Streptokinase 1500000, IU/5ml, Dose: 30000 IU in 100µl.

Procedure: In vitro clot lysis activity of the leaves was carried out according to the method with minor modifications. With ethical considerations, and aseptic precaution, 5 ml of venous blood was drawn from healthy volunteers (n = 3) having no history of smoking, taking lipid lowering drugs, oral contraceptive or anticoagulant therapy and transferred to different pre weighed sterile micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were subjected to incubation at 37°C for 45 min. After the formation of clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot

(clot weight = weight of clot containing tube – weight of tube alone).

To each micro-centrifuge tube containing pre-weighed clot, 100 µl solution of different extracts, concentration 1 mg/mL, were added accordingly. As a positive control, 100 µl of streptokinase and asn a negative non thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 min and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption. At last, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

% of clot lysis = (wt. of lysis clot /initial clot wt.) ×100 [21,22],

Results

Cytotoxicity evaluation:

The lethal concentration (LC50) of the test both extracts after 24 hours were found by a plot of percentage of the shrimps died against the logarithm of the extract's concentration as well as the best fit line was found from the curve data by means of regression analysis. Vincristine sulphate are used as a standard positive control and the LC50 compared with negative control.

The LC50 of the leaf, stem extract and standard are 71.70, 55.0 and 2.63 µg/ml; respectively whereas The LC90 of the leaf, stem extract and standard are 633.50, 532.33 and 5.73 µg/ml; respectively.

Thrombolytic activity assay

Table-3 indicates the results in which 100 µl SK, a positive control (30,000 I.U.), was used for comparison leaf and stem extract of *Rhynchotechum ellipticum* (Gesneriaceae). was shown 41.98 and 34.21 % clot lysis, compared to control; respectively. Streptokinase used as the standard was shown 68.42 % clot lysis as well.

Discussion

The positive control (vincristine sulphate), the cytotoxicity exhibited by the leaf and stem extracts of the plant has been shown activity. This evidently introduces the presence of bioactive principles in these both extracts which may be very necessary as antiproliferative, antitumor, pesticidal aswell as other bioactive agents [23]. Platelets play an essential role in the process of formation of thrombus by adhering to be damaged regions of the endothelial surface. The activated platelets form platelets to platelets bonds and apart from bind to the leucocytes and bring off them into a perplex method of plaque formation as well as growth [24]. Streptokinase forms a 1:1 stochiometric complex with plasminogen which is cpable converting additional plasminogen to plasmin [25].

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Table 1 : Chemical group tests of *Rhynchotechum ellipticum* (Gesneriaceae)

Tested groups	Leaf extract	Stem extract
Tannins	+	+
Phenols	-	+
Flavonoids	+	-
Saponins	-	+
Terpenoids	+	+
Gum	+	+
Alkaloids	+	+
Glycosides	+	+

(+) Indicates presence, (-) Indicates absence.

Table 2: Test result of the cytotoxic activity of different acetone extracts of Leaf and Stem

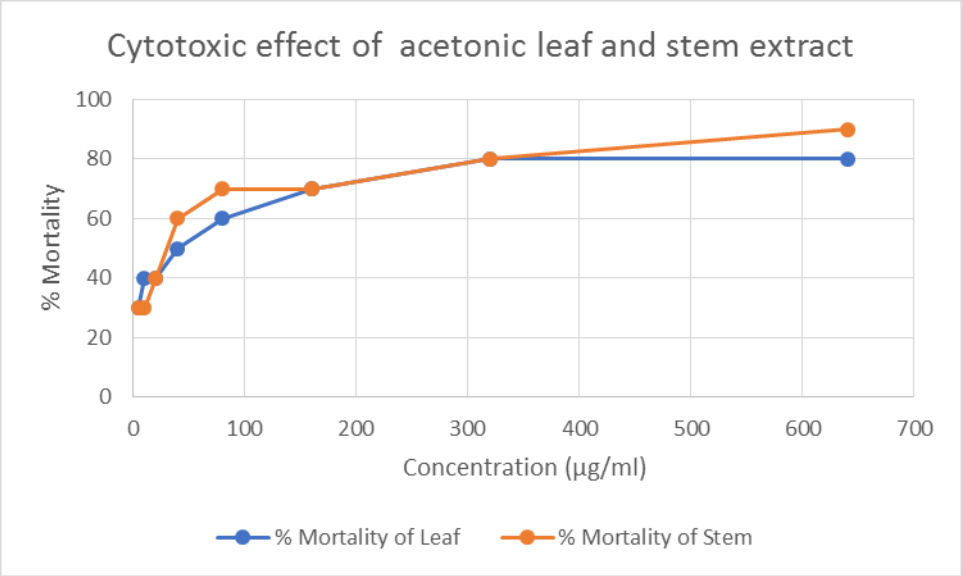
Treatment	Conc.(µg/ml)	No. of nauplii taken	No. of dead nauplii	% Mortality	LC50 (µg/ml)	LC90 (µg/ml)
Leaf extract	640	10	8	80	71.70	633.50
	320	10	8	80		
	160	10	7	70		
	80	10	6	60		
	40	10	5	50		
	20	10	4	40		
	10	10	4	40		
	5	10	3	30		
Stem extract	640	10	9	90	55	532.33
	320	10	8	80		
	160	10	7	70		
	80	10	7	70		
	40	10	6	60		
	20	10	4	40		
	10	10	3	30		
	5	10	3	30		
Vincristine Sulphate	5	10	10	100	2.63	5.73
	2.5	10	9	90		
	1.25	10	8	80		
	0.625	10	6	60		
	0.315	10	5	50		
	0.156	10	4	40		
	0.078	10	3	30		

Table 3: Thrombolytic activity test.

Sample	Wt. of Blank tube (g)	1st clot + tube (g)	1st clot	2nd clot + tube (g)	2nd clot	Lysis weight (g)	% of lysis
SK	0.83±0.01	1.79±0.006	0.95±0.02	1.26±0.05	0.32±0.01	0.65±0.02	68.42
DW	0.83±0.01	1.48±0.01	0.66±0.01	1.43±0.01	0.62±0.01	0.05±0.06	7.58
Leaf	0.82±0.01	1.67±0.02	0.81±0.06	1.33±0.01	0.46±0.01	0.34±0.01	41.98
Stem	0.82±0.01	1.61±0.01	0.76±0.01	1.32±0.006	0.52±0.01	0.26±0.006	34.21

SK=Streptokinase as a standard reference, DW=Distill Water as control, Values are represented as Mean±SD.

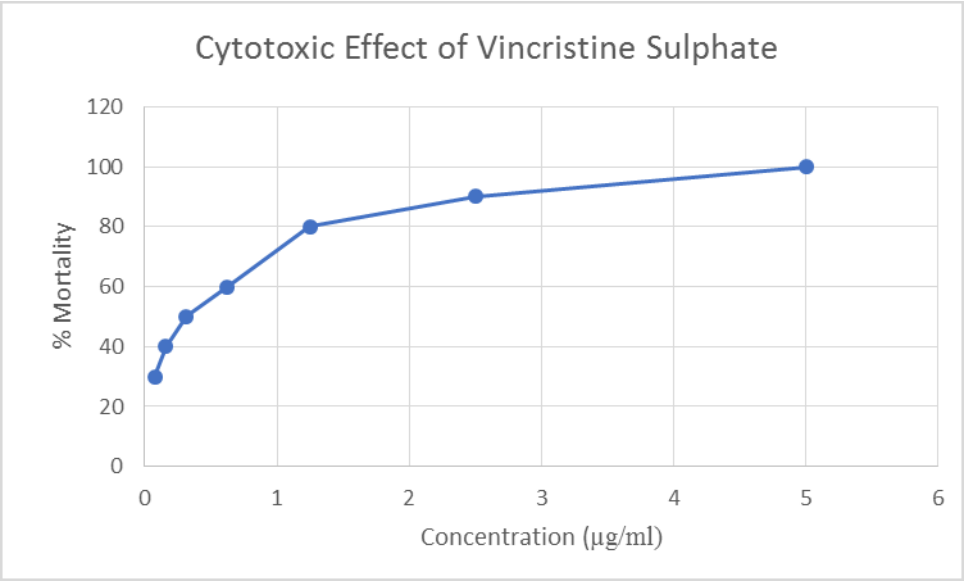
Figure 1



Linear equation: y= 0.0712x+44.895; R2=0.6727 [Leaf extract]

Linear equation: y= 0.0838x+45.391; R2=0.6534 [Stem extract]

Figure 2



Linear equation: y= 12.917x+45.979; R2= 0.7707

Both figures comparison between the cytotoxic effect of samples and standard.