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PRELIMINARY PHYTOCHEMICAL SCREENING, IN VITRO THROMBOLYTIC AND MEMBRANE STABILIZING ACTIVITIES OF GYNURA NEPALENSIS LEAVES OF BANGLADESHI ORIGIN

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ABSTRACT

Gynura nepalensis (Family: Asteraceae), is one of the commonly used medicinal plants that was investigated for *in vitro* thrombolytic and membrane stabilizing activity. People of Bangladesh have been using this herbaceous plant for various medicinal purposes from the treatment of diabetes mellitus to control of blood pressure etc. Preliminary Phytochemical screening influenced the presence of different phytoconstituents e.g. alkaloids, glycosides, tannins, carbohydrates, phenols, resins and coumarins. The crude ethanolic extract of the leaves of G. nepalensis and its different partitionates were studied by using Streptokinase (SK) and acetyl salicylic acid (ASA) as standards for thrombolytic and membrane stabilizing activities. In case of in vitro thrombolytic activity, the ethanolic fraction of leaves (EF) Showed 50.84 ± 0.79 %, n-Hexane fraction (HXF) 47.70± 0.43 %, chloroform soluble fraction (CF) 42.94± 0.13%, aqueous soluble fraction (AQF) 41.15± 0.92% and pet-Ether soluble fraction (PF) 24.98 ± 0.35% against thrombosis whereas standard SK showed 64.19 ± 0.22%. ASA exhibited 71.25 ± 0.21% haemolysis inhibition of human red blood cell (RBC) membrane in normal condition whereas EF inhibited 64.79 ± 0.51%, HXF inhibited 58.63 ±0.36%, PF inhibited 51.98 ±0.12%, CF inhibited 47.42 ±0.22%, and AQF 44.51 ±0.10%. In heat induced condition, the EF inhibited 54.95±0.42%, HXF inhibited 50.57±0.79%, CF inhibited 44.92±0.24%, PF inhibited 48.90±0.71%, and AQF 39.93±0.46% respectively and ASA showed 61.64±0.31% for against unstable membrane. The abovementioned results suggested that the ethanolic extracts exhibit favourable influence for the use of this plant as traditional medicine and its further study.

KEYWORDS: *Gynura nepalensis*, Asteraceae, thrombolytic, membrane stabilizing activity.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies (Kumar et al., 2005). 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 nature of medicinal plants are most of the presence of various chemical substances of different composition (*Jahan et al*., 2017). *Gynura nepalensis* is a medicinal plant having wide curative application *based on ethnomedical knowledge*. Many researchers, having recognized this wide potential, have conducted studies on *G. nepalensis* (Salas, 2016 and San Andres, 2014). Forty-four species of Gynura are distributed in Africa, South and East Asia and Australasia Vanijajiva, 2009; Vanijajiva & Kadereit, 2011). The leaf extract of *G. nepalensis* is one of the 40 ethnomedicinal plants used to treat indigestion by the members of the Apatani tribe, in the Ziro Valley of Arunachal Pradesh, one of the 28 states in Northeast India and also considered the 12th mega bio-diverse regions of the world (Kala, 2005). The leaves are also used to treat diabetes that has been reported in Bangladesh (Afroz, Uddin, & Hassan, 2014) and in the Philippines (Ursulom & Rialubin, 2013) and *G. nepalensis*'s juice is used to heal cuts and wounds in Nepal (Manandhar, 2002, cited in Afroz, Uddin, & Hassan, 2014) and external wounds in the Philippines (Ursulom & Rialubin, 2013). Studies have shown that plants have chemical components and biological activities that pro-duce definite physiological actions in the body and therefore, could be used to treat various ailments. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al., 2005). The present study reported biological activity of the extract of ***Gynura nepalensis*** and its fractions on blood cells exposed to both heat and hypotonic induced lyses and with it the thrombolytic activity of the plant was also evaluated.

MATERIALS AND METHODS

Plant materials

Fresh leaves of ***Gynura nepalensis*** were collected in June 2017, from Dhaka city. Plant identification and authentication was done by Bangladesh National Herbarium, Mirpur, Dhaka, Accession no is 45113. The leaves were shade dried and then oven dried to facilitate grinding and then stored in a air tight container.

Preparation of extract

The dried powder materials (about 300 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. Then, the whole mixture was subjected to filter by **clean, white cotton** materials. Then the filtrate was filtered again using Whatman filter paper, total filtrate was concentrated using a rotatory evaporator to get the crude extract. The partition of the concentrated aqueous ethanol extract was done by the Kupchan method and the resultant partitionates, i.e. Chloroform, Petroleum ether, N-hexane 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 secondary metabolites (Trease and Evans, 2002; Oyedapo et al., 1999) 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 and phenols with Ferric chloride and Potassium dichromate solutions, Terpenoids with the use of chloroform and conc. H_2SO_4 and steroids with Salkowski reagent, Tannins with Broymers test, reducing sugars with Benedict's reagent etc. (Table1).

Streptokinase (SK)

Commercially available lyophilized Altepase (Streptokinase) vial was collected and 5 ml 0.9% NaCl was added and mixed properly. This suspension was used as a stock for *in vitro* thrombolysis activity.

Blood sample

Blood was drawn from five healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and was transferred to the previously weighed sterile Eppendorf tubes.

Thrombolytic activity

Sample preparation

For sample **preparation,** the crude extract was suspended in 10 ml of distilled water and was shaken vigorously on a sonicator. The suspension was kept overnight and decanted to remove soluble supernatant, which was filtered through a filter paper. The solution was then ready for *in vitro* evaluation.

Thrombolytic assay

Following the method developed by Prasad *et al.* (2007) *w*hole 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. The serum was completely removed after clot formation without interfering 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. 100 µl of streptokinase (SK),as a positive control, and 100 µl of distilled water, as a negative non thrombolytic control were separately added to the control Eppendorf tubes. All tubes were labeled carefully and then incubated at 37°C for 90 minutes and observed for clot lysis. The released fluid was removed again 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:

% 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*. (2008) The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat induced RBC haemolysis.

Hypotonic solution Induced hemolysis

For this experiment, erythrocyte (RBC) suspension (0.5ml) was taken with 5ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffer having pH 7.4. The extractive was taken at a concentration of (2mg/ml) and acetyl salicylic acid (0.1mg/ml) to the mixtures. The acetyl salicylic acid was used as reference standard. The mixtures were centrifuge for about 10 min at 3000 rpm, and incubated for 10 min at a room temperature. The absorbance of supernatant portion was measured at 540 nm using UV spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was calculated out using the following equation.

% inhibition of haemolysis $=100\times \{(OD_1-OD_2)/OD_1\}$

Where, OD1 = optical density of hypotonic-buffered saline solution (control) alone and

OD2 = optical density of test sample in hypotonic solution.

Heat induced haemolysis

Isotonic buffer solution containing 2mg/ml of different fractions of ***Gynura nepalensis*** were taken into two duplicates of centrifuging tube. The vehicle in the same amount was added to another tube as control. Erythrocyte suspension (30 µl) was added to each tube and was mixed gently. One pair of tubes was incubated at 54°C for 20 min in a water bath and the other pair was maintained of 0-5°C in an ice bath. The reaction mixture

was centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant portion was measured at 540 nm. The percentage of inhibition was calculated out according to the equation:

% inhibition of haemolysis = $100\times(1-100\times OD_2-OD_1/OD_3-OD_1)$

$= 100\times(OD_3-OD_2/OD_3-OD_1)$

RESULTS AND DISCUSSION

Thrombolytic activity

As a part of discovery of cardioprotective drugs from natural sources the extractives of *Gynura nepalensis* were assessed for thrombolytic activity and the results are presented in table 2. Addition of 100 µl Streptokinase (30,000 I.U.), standard to the clots along with 90 minutes of incubation at 37°C, showed 64.19± 0.22% clot lysis. In this study, the ethanol extract of *Gynura nepalensis* showed thrombolytic activity of 50.84 ± 0.79%, pet. ether soluble fraction 24.98 ± 0.35%, N hexane soluble fraction 47.70± 0.43%, chloroform soluble fraction 42.94± 0.13% and aqueous soluble fraction showed 41.15± 0.92% lysis.

Membrane stabilizing activity

Different partitionates of *Gynura nepalensis* at the concentration of 2 mg/ml were tested against lysis of human erythrocyte membrane induced by hypotonic solution and heat as compared to standard Acetyl salicylic acid (0.1 mg/ml). The results are presented in table 3. Among the different fractionates, the ethanol extract inhibited 64.79 ± 0.51 and 54.95±0.42 of haemolysis of RBC induced by hypotonic solution and heat as compared 71.25 ±0.21and 61.64±0.31 demonstrated by acetyl salicylic acid, PF 51.98 ±0.12 and 48.90±0.71, HXF 58.63 ±0.36 and 50.57±0.79, CF 47.42±0.22 and 44.92±0.24, AQF 44.51±0.10 and 39.93±0.46.

Table 1: Results of different chemical groups of the ethanolic extract of *Gynura nepalensis*.

Plant extract	alkaloids	tannins	flavonoids	Steroids	saponins	phenols	glycosides	resins	Carbohydrates	Coumarins
Ethanolic extract	+	+	-	-	+	+	+	+	+	+

(+) means positive, (-) means negative

Table 2: % Clot lysis by different extracts of ***Gynura nepalensis.***

Fraction	Weight of empty vial (w1) g	Weight of vial with clot (w2) g	Weight of clot before lysis (w2-w1) g	Weight of vial with clot after lysis (w3) g	Weight of clot lysis (w2-w3) g	% of clot lysis
EF	6.27	6.63	0.36	6.44	0.19	50.84 ± 0.79
PF	6.10	6.50	0.40	6.40	0.10	24.98 ± 0.35
HXF	6.76	7.11	0.36	6.94	0.17	47.70± 0.43
CF	6.70	7.27	0.56	7.02	0.24	42.94± 0.13
AQF	6.66	7.07	0.41	6.90	0.17	41.15± 0.92
Blank	5.17	5.73	0.56	5.70	0.03	5.36 ± 0.15
SK	4.64	5.03	0.39	4.78	0.25	64.19± 0.22

Table 3: Effect of different extracts of *Gynura nepalensis* on hypotonic solution & heat induced hemolysis of erythrocyte.

Fraction	% inhibition haemolysis	
	Hypotonic solution induced	Heat induced
EF	64.79 ± 0.51	54.95±0.42
PF	51.98 ±0.12	48.90±0.71
HXF	58.63 ±0.36	50.57±0.79
CF	47.42 ±0.22	44.92±0.24
AQF	44.51 ±0.10	39.93±0.46
ASA	71.25 ±0.21	61.64±0.31
Hypotonic solution	--	--

Values are expressed as mean ± SD (standard deviation).EF=ethyl soluble fraction, HXF= hexane soluble fraction,CF= chloroform soluble fraction, AQE=aqueous soluble fraction,ASA=acetyl acetic acid

CONCLUSION

It is evident that the ethanolic crude extracts of the leaves of *Gynura nepalensis* has significant in vitro thrombolytic and membrane stabilizing activity obtained from the results of the above study. Besides this preliminary study, the extracts should be thoroughly checked for phytochemical and pharmacological activity to find out more specific medicinal and pharmaceuticals potentials as well as to isolate and characterize the specific active ingradients responsible for the presented activities.

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