Bioactivities and Chemical Profiling of *Sesbania grandiflora* (L.) Poir. Leaves Growing in Bangladesh

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ABSTRACT: The ethanolic extract of leaves of *Sesbania grandiflora* and their organic and aqueous soluble partitioning substances were evaluated for thrombolytic and membrane stabilizing potentials *in vitro*. In thrombolytic assay, among all partitionates, the ethyl acetate soluble fraction showed highest percent of clot lysis (59.57%) as compared to 69.23% and 3.07% exhibited by the standard streptokinase and water (negative control), respectively. With respect to the membrane stabilizing activity, ethyl acetate soluble fractions of *S. grandiflora* also profoundly inhibited the hemolysis of erythrocytes induced by osmosis (64.30 ± 0.64%) and heat (57.21 ± 0.69%), respectively. In contrast, standard acetyl salicylic acid resulted in 70.12 ± 26% inhibition of osmotically-induced hemolysis and slightly higher level of protection against heat-induced hemolysis (73.90 ± 0.29%). The ethanol extract of leaves of *S. grandiflora* revealed significant percentage of thrombolytic and membrane stabilizing activities.

Key words: *Sesbania grandiflora*, thrombolytic, membrane stabilizing.

INTRODUCTION

Sesbania grandiflora (L.) Poir. belongs to the family Fabaceae and locally known as 'Bakful'. It is a species of tropical climate, short lived, quick growing and soft wooded tree.[1] Almost every single part of *S. grandiflora* is used as folkloric or traditional medicine to treat many diseases such as dysentery, stomatitis, fever, small pox, sore throat, headache etc. The dried leaves are often used to make tea and are considered to have good antibacterial, anthelmintic, antitumor and contraceptive properties.[2] A poultice made from the leaf juice is used in folkloric system as effective treatment for bruises.[3] *S. grandiflora* leaves and flowers provided sterols, saponins and tannins. These bioactive constituents have potential health benefits such as antibacterial, antifungal, antioxidant, antiurolithiatic, anticonvulsant, anxiolytic and hepatic protective properties.[4] Based on the previous ethnobotanical literatures and traditional medicinal values as judged by local users and healers, we studied the organic and aqueous soluble fractions of ethanolic extract of *S. grandiflora* leaves for thrombolytic and membrane stabilizing activities.

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MATERIALS AND METHODS

Plant materials. The leaves of *S. grandiflora* were collected from Tangail district, Bangladesh in February 2015 and were identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen (DACB accession no. 42103) for this plant has also been preserved in Bangladesh National Herbarium.

Preparation of extract. The collected leaves were shade dried for several days and dehydrated at 37 °C overnight to facilitate crushing by an electrical grinder. The coarse powder (400gm) was dipped in 2000 ml of 90% ethyl alcohol. The filtrate was condensed by a vacuum rotary evaporator at 40°C. Concentrated aqueous ethanolic extractive was fractionated by the modified Kupchan method[5] to yield pet. ether (PSF), carbon tetrachloride (CTSF),

chloroform (CSF), ethyl acetate (EASF) and aqueous (AQSF) soluble fractions.

Phytochemical screenings. The freshly prepared organic extracts were qualitatively tested for the presence of various phytochemicals. These were identified by characteristic color changes using standard procedures, previously described by Sofowara.[6]

Determination of total flavonoid content. To determine total flavonoid content of the extractives, aluminium chloride colorimetric method was used as developed by Chang *et al.*[7] In brief, 50 µL of extract of CEE, EASF, PSF, CTSF and CSF (1 mg/mL ethanol), was mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution, 0.3 mL of 10% $AlCl_3$ solution were added. After 5 min of incubation, the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was adjusted to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. A blank sample was prepared also. A standard curve was extrapolated by taking optical absorbance using different concentrations of standard quercetin solution at similar conditions.

Determination of total phenolic content. The whole phenols were assayed according to the method developed by Dewanto *et al.*[8] Concisely, 0.3 ml (300 µl) of the CEE and other extractives were taken into separate volumetric flask (10 ml) containing 2.7 ml of Folin-Coicalteu (1:10) phenol mixture. Five minutes later, 2 ml of 7.5 % sodium carbonate solution was poured to every test tube and shaken well. Then they were stored at room temperature for 30 min in dark place after warming at 45 °C. A series of reference standard solutions of gallic acid were treated similarly without the extract. Absorbance for test and standard solutions were estimated at 725 nm with an UV/Visible spectrophotometer against the blank. The entire phenol content was determined from extrapolation of gallic acid standardization curve.

***In vitro* thrombolytic activity.** The crude extract and other fractions were individually suspended in 1 ml of sterile distilled water and shaken vigorously on a sonicator to obtain a suspension. The suspensions of the extract were kept overnight and decanted later to remove soluble supernatant, which was filtered through a filter paper to obtain residueless solution. The resulting solution was used as such for *in vitro* evaluation of clot lysis activity.

The thrombolytic activities of the various fractions were evaluated by the modified Daginawala method.[9] Finally, differences obtained in weight were taken and clot lysis was expressed as:

% of clot lysis = (wt. of released clot /clot wt.) × 100.

***In vitro* membrane stabilizing assay.** The membrane stabilizing activity of the extract was assessed by evaluating their ability to inhibit the breakdown of human red blood cells, provoked osmotically by hypotonic solution or heat by following the method developed by Omale.[10]

Hypotonic solution-induced hemolysis. The test sample containing stock erythrocyte (RBC) suspension (0.5 ml) was mixed with 5 ml hypotonic solution (50 mM NaCl in 10 mM sodium phosphate buffered saline, pH 7.4), ethanolic extract fraction (2 mg/ml) each and acetyl salicylic acid (0.1 mg/ml) in different centrifuge tubes. The acetyl salicylic acid was used as the reference standard. The mixtures were centrifuged for 10 min at 3000 rpm, and incubated for 10 min at 25 °C. The absorbance of supernatant was measured at 540 nm using UV/Visible spectrophotometer.

Heat-induced haemolysis. The pair of centrifuge tubes containing 2 mg/ml of crude extract and fraction (EASF, CSF, PSF, CTSF, AQSF) solutions and 5 ml of buffered isotonic NaCl solution were taken. A pair of centrifuge tubes were prepared by using 5 ml of isotonic buffered solution plus ASA at a concentration of 0.1 mg/ml for positive control and negative control containing only 5 ml of the isotonic buffered solution. Erythrocyte suspension (30 µl) was added to all tube and mixed gently by inversion. One set of tubes was incubated in a water bath at 54 °C for 20 min. The other set of tubes were maintained in

an ice bath (0-5 °C). At the end of the incubation period, the samples were centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant was measured at 540 nm.

Statistical analysis. Three replicates of each sample were used for each test to facilitate statistical analysis and the data are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The crude ethanol extracts of leaves of *S. grandiflora* and their Kupchan partitionates were used to determine the content of bioactive compounds and justify the traditional uses in thrombosis and inflammatory disorders.

Phytochemical screenings. The qualitative phytochemical screenings revealed the presence of alkaloids, flavonoids, and phenols in the fractions. The same for carbohydrates was observed with the exception of PSF. Reducing sugar was detected only in EASF and CSF, and steroids only in PSF.

Total flavonoid and phenol content. The total phenol and flavonoid contents are shown in table 1.

Table 1. Total flavonoid and tannin content of CEE and extractives of *S. grandiflora*.

Crude extracts/ Fractions	Total flavonoid (mg of QAE /gm of dry extract)	Total phenol (mg of GAE/gm of dry extract)
EASF	57.37 ± 0.15	47.08 ± 0.22
CTSF	17.18 ± 0.12	13.18 ± 0.10
CSF	51.76 ± 1.80	30.09 ± 0.11
PSF	44.44 ± 0.37	39.47 ± 0.33
CEE	-	18.38 ± 0.49

Results are expressed as mean ± SD (n = 3), are statistically significant (P < 0.05), CEE = Crude ethyl extract

Thrombolytic activity. The extractives of leaves of *S. grandiflora* were evaluated for thrombolytic activity to determine the ability of clot lysis by using a positive control (SK) that showed 69.23% lysis of clot. On the other hand sterile distilled water, a negative control, exhibited a negligible percentage of lysis of clot 3.07%. The percentage of clot lysis by various extracts were observed in the following order, EASF (59.57%), PSF (57.40%), CSF (51.5%), CEE (33.18), CTSF (24.80), AQSF (23.80%). So, it is clear that significant (p value < 0.001) percentage of thrombolytic activity was exhibited by all extractives of *S. grandiflora* as presented in table 2. Thrombolytic agent like SK is used as break already formed blood clots in clinical settings where ischemia may be fatal (acute myocardial infarction, pulmonary embolism, ischemic stroke, and arterial thrombosis).[11] Our findings showed that the thrombolytic activities of at least four of our test samples (EASF, PSF, CTSF and CSF) revealed positive results in comparison to positive and negative controls. The results from the study clearly indicated that *S. grandiflora* extract is a potential clot lysis agent.

Table 2. Percentage (%) inhibition of heat- and hypotonic-solution induced haemolysis of erythrocyte membrane and thrombolytic activity of different fractions of *S. grandiflora*.

Samples	% Inhibition of haemolysis		% of clot lysis
	Heat- induced	Hypotonic solution- induced	
EASF	57.21 ± 0.69*	64.30 ± 0.64**	59.57**
CSF	44.23 ± 0.57	48.65 ± 0.77*	51.5**
PSF	52.30 ± 0.61	51.30 ± 0.64*	57.40**
AQSF	31.34 ± 0.42	28.34 ± 0.42	34.11*
CTSF	41.14 ± 0.42	38.31 ± 0.51*	24.80*
ASA	70.12 ± 0.26*	73.90 ± 0.29*	-
SK	-	-	69.23**
CEE	-	-	33.18*
Negative control	-	-	3.07*

Values are expressed as mean ± SD (standard deviation); *P < 0.005, **P < 0.001; statistically significant as compared to positive control and negative control, SK = Streptokinase.

Membrane stabilizing activity. The extractives of *S. grandiflora* significantly protected the haemolysis of human RBC membrane induced by hypotonic solution and heat as compared to the standard ASA. The membrane stabilizing activity against hypotonic solution induced stabilization was observed in the following order ASA (73.90 ± 0.29%), EASF (64.30% ± 0.64), PSF (51.30 ± 0.64%), CSF (48.65 ± 0.77) CTSF (38.31 ± 0.51) and AQSF (31.30% ± 0.51). In heat -induced method, the values of ASA (70.12 ± 0.26), CSF (44.23 ± 0.57 %)

and EASF (57.21 ± 0.69%) were decreased slightly but for AQSF (31.34% ± 0.42) and PSF (52.30 ± 0.61) a little increase in membrane stabilizing activities was noted (Table 2). The possible mechanisms for the membrane stabilizing effect of *S. grandiflora* leaves were not known. However, a number of studies have shown that flavonoids and other phenolic compounds which exhibit analgesic and anti-inflammatory effects also display membrane stabilizing activity.[12] The presence of significant amount of compounds in the extract of *S. grandiflora* revealed that the leaves have potential to inhibit hemolysis of RBCs induced by hypotonic solution and heat.

CONCLUSIONS

Phytochemical studies of the *S. grandiflora* leaf extractives demonstrated the presence of significant amount of bioactive compounds. This ensures that the plant possess aforesaid pharmacological potentials. On the basis of our investigations, the folkloric uses of *S. grandiflora* leaves as potential thrombolytic and membrane stabilizing activities are justified.

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