Thrombolytic, Membrane Stabilizing and Hypoglycemic Activities of *Anethum sowa* Linn.

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ABSTRACT: The hexane (HE), dichloromethane (DCME), ethyl acetate (EAE) and methanol (ME) extracts of seed and stem of *Anethum sowa* were subjected to screenings for thrombolytic, membrane stabilizing and hypoglycemic activities. Ethyl acetate extract of stem showed highest thrombolytic activity. In addition, the dichloromethane, ethyl acetate and methanol extract of seed revealed higher percentage (%) of inhibition in hypotonic solution induced hemolysis. In hypoglycemic activity, the dichloromethane and ethyl acetate extracts of stem and seed displayed significant blood glucose lowering effect.

Key words: *Anethum sowa* Linn., thrombolytic, membrane stabilizing, hypoglycemic activity.

INTRODUCTION

Traditional systems of medicine continue to be widely practised on many accounts. Side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as source of medicines for a wide variety of human ailments due to a rich resources of bioactive components which can be used in drug development either pharmacopoeial, nonpharmacopoeial or synthetic drugs due to their therapeutic values.[1,2] In addition, Bangladesh is a good repository of medicinal plants belonging to various families. This is why, appropriate scientific proofs are essential to explore for the discovery and development of newer and safer drug candidates for treating diverse diseases.

Anethum sowa Linn. (Common name- Dill; Bengali- Shulfa; Family-Apiaceae), is an annual or a biennial cold weather glabrous and aromatic herb. In Bangladesh it is abundantly cultivated in the northern parts of the country and throughout India mainly in Punjab, Uttar Pradesh, Gujarat, Maharasshtra, Assam and West Bengal. It is often found with weed of cultivation and even as an escape in irrigated fields.[3] Seeds are used as insecticidal, ovicidal and has synergistic activity of dillapiol and also contains essential oil having antioxidant and antimicrobial activities.[4-7]

There are very few reports that describe the scientific justification supporting the traditional use of seed and stem of *A. sowa*. Therefore, the present study was undertaken to investigate the thrombolytic, membrane stabilizing and hypoglycemic activities of seed and stem of *A. sowa* plant using *in vitro* and *in vivo* models.

MATERIALS AND METHODS

Plant collection, identification and authentification. Fresh stem and seeds of *A. sowa* were collected from BCSIR campus, Dhaka and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB No. 31282) has been deposited.[3]

Extraction and processing. Freshly collected stem and seed of *A. sowa* were dried in open air and

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powdered by using a grinding machine. The air-dried and powdered material of seed (1.5 kg) was firstly soaked in *n*-hexane (2.5 litres x 3), dichloromethane (2.5 litres x 3), then ethyl acetate (2.5 litres x 3) and finally in methanol (2.5 litres x 3) at room temperature for 2 days each soaking. Consequently gummy mass of *n*-hexane extract (10.8 g), dichloromethane extract (7.4 g), ethyl acetate extract (12.1 g) and methanol extract (26.4 g) were obtained from filtrate of the respective solvent using rotary evaporator under reduced pressure. By the same process, the air-dried and powdered material of stem (1.5 kg) was firstly soaked in *n*-hexane (2.5 litres x 3), dichloromethane (2.5 litres x 3), ethyl acetate (2.5 litres x 3) and finally in methanol (2.5 litres X 3) at room temperature for 2 days. Consequently gummy mass of n-hexane extract (7.2 g), dichloromethane extract (5.8 g), ethyl acetate extract (8.7 g) and methanol extract (15.9 g) were found from filtrate of respective solvent using rotary evaporator under reduced pressure.[3] The HE, DCME, EAE and ME extracts of seed and stem of *A. sowa* were subjected to studies for thrombolytic, membrane stabilizing and hypoglycemic activities.

***In vitro* thrombolytic activity.** The crude extract was mixed with 10 ml of sterile distilled water and 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 of clot lysis activity**.** The thrombolytic activity of the prepared extracts was evaluated by the method of Daginawala, using streptokinase (SK) as a standard drug.[8] Venous blood (5 ml) was drawn from healthy volunteers, and transferred in different pre-weighed sterile tubes (0.5 ml/tube) to form clots and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot formed and each tube having clot, was again weighed to determine the clot weight [clot weight = weight of clot containing tube – weight of tube alone]. The tubes containing pre-weighed clot were properly labeled and 100 µl crude extract as well as aqueous solution of different fractionates were added to the tubes separately. As a positive control, 100 µl of SK and 100 µl of isotonic (a negative non thrombolytic control) solution were separately added to two clot containing tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and vials were again weighed to observe the difference in weight after clot disruption. Differences 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

***In vitro* membrane stabilizing activity.** The membrane stabilizing activity of the extractives was determined on human erythrocytes by the method of Omale.[9]

Hypotonic solution-induced hemolysis. The experiment was carried out with hypotonic solution. Stock erythrocyte (RBC) suspension (0.5 ml) and 4.5 ml hypotonic solution in 10 mM Na phosphate buffer saline (pH 7.4) containing different extracts (2.0 mg/ml) or acetyl salicylic acid (0.10 mg/ml) were mixed. The mixtures were centrifuged for 10 min at 3000 rpm, and incubated for 10 min at temperature of 25°C. The absorbance of supernatant content haemoglobin was measured at 540 nm using UV Vis spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

% Inhibition of haemolysis =100× $\{(OD_1-OD_2)/OD_1\}$ where, OD_1= optical density of hypotonic buffered saline solution alone (control) and OD_2 = optical density of the test sample in hypotonic solution.

***In vitro* hypoglycemic activity.** The glucose tolerance test (GTT) is one of the most acceptable methods to evaluate the hypoglycemic activity.[10] Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resources Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (icddr,b) were used for the experiment. Fifty experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III (A-B), group-

IV (A-B), and group V (A-B) and group-VI (A-B) consisting of 3 mice in each group. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. In order to administer the extract at doses of 200 mg/kg body wt and 400 mg/kg body wt of mice, the exactly weighed extracts were measured and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of standard (Glibenclamide) at the dose of 5 mg/kg body weight, 10 mg tablet was dissolved into 3.0 ml normal saline (0.9% NaCl). After 60 min, all groups were treated with 10% glucose solution. After 120 and 180 min of glucose loading, blood samples were collected from tail vein. By using glucometer blood glucose level was measured.

RESULTS AND DISCUSSION

The *in vitro* thrombolytic activity study revealed that the addition of 100 µl streptokinase (SK, 30,000IU) to the clots and subsequent incubation for 90 minutes at 37°C showed 66.77% lysis of clot. On the other hand, sterile distilled water, a negative control, exhibited a negligible percentage of lysis of clot (2.64%). The percentage of clot lysis with *n*-Hexane extract (HE), dichloromethane extract (DCME), ethyl acetate extract (EAE), methanol extract (ME) of stem were 15.13, 9.40, 45.3 and 38.44%, respectively and 16.36, 13.38, 43.77 and 41.32%, respectively in case of seed**.** In this study, thrombolytic activity of all the extracts was evaluated as a part of discovery of cardioprotective drugs. In addition, it was observed that among all the fractions ethyl acetate of stem showed highest percentage of clot lysis.

Table 1. Thrombolytic activity of stem and seed extracts of *A. sowa*.

Sample	Thrombolytic activity (% of clot lysis)	
	A. sowa stem	*A. sowa* seed
HE	15.13 ± 0.59	16.36 ± 1.44
DCME	9.40 ± 0.98	13.38 ± 1.03
EAE	45.3 ± 2.05*	43.77 ± 2.01*
ME	38.44 ± 1.98	41.32 ± 2.03*
Standard (Streptokinase)	66.77 ± 0.66	
Blank	2.64 ± 0.22	

Data are expressed as mean ± SD (n = 3). *****p < 0.0001 indicates extremely significant compared to control. HE: *n*-hexane extract, DCME: dichloromethane extract, EAE: ethyl acetate extract, ME: methanol extract.

Table 2. Percentage (%) inhibition of hypotonic solution induced hemolysis of stem and seed of *A. sowa*.

Sample	Hypotonic solution induced hemolysis (% of inhibition)	
	A. sowa L. stem	*A. sowa* L. seed
HE	64.30 ± 0.46	57.14 ± 0.88
DCME	61.29 ± 0.85	82.3 ± 1.03
EAE	59.65 ± 1.65	92.4 ± 1.01
ME	67.51 ± 1.88	79.11 ± 2.01
Acetyl salicylic acid	77.91 ± 0.29	

Data are expressed as mean ± SD (n=3). HE: *n*-hexane extract, DCME: dichloromethane extract, EAE: ethyl acetate extract, ME: methanol extract.

Table 3. Hypoglycemic activity of stem of *A. sowa.*

Sample	Hypoglycemic activity (*A. sowa* stem) (mmol/liter)							
	200 mg/kg				400 mg/kg			
	0 min	60 min	120 min	180 min	0 min	60 min	120 min	180 min
HE	5.6±1.45	5.4±1.36	5.5±1.43	5.1±1.74	5.3±1.23	5.3±1.32	5.1±1.45	4.9±1.41
DCME	4.1±1.69	3.9±0.93	3.7±1.34	3.5±0.73	4.2±1.13	3.9±1.15	3.5±1.54	3.1±1.32*
EAE	4.2±1.41	3.8±1.40	3.7±1.36	3.4±1.13	4.1±1.30	4.0±1.03	3.7±1.14	3.2±1.56*
ME	4.9±1.78	4.6±1.08	4.3±1.32	4.0±1.66	5.1±1.32	5.0±1.35	4.8±1.61	4.6±1.52
Control (Placebo)					5.9±1.14	8.6±0.79	6.0±1.10	5.8±1.33
Standard (5 mg/kg Glibenclamide)					4.6±1.60	3.7±1.34	3.1±1.09	2.7±1.43

Data are expressed as mean ± SD (n=3). *****p = 0.06 indicates "statistically significant" compared to control. HE: *n*-hexane extract, DCME: dichloromethane extract, EAE: ethyl acetate extract, ME: methanol extract.

Table 4. Hypoglycemic activity of seed of *A. sowa*.

Sample	Hypoglycemic activity (*A. sowa* seed) (mmol/liter)							
	200 mg/kg				400 mg/kg			
	0 min	60 min	120 min	180 min	0 min	60 min	120 min	180 min
HE	5.7±1.55	5.3±1.61	5.6±1.21	5.0±1.34	5.4±1.10	5.5±1.03	5.3±1.56	5.1±1.48
DCME	4.7±1.09	4.0±1.46	3.8±0.56	3.3±1.45	4.4±1.32	4.2±1.21	3.6±1.34	3.3±1.03*
EAE	4.3±1.31	3.7±1.41	3.6±0.89	3.2±1.02	4.5±0.41	4.0±1.12	3.8±1.30	3.3±1.64*
ME	5.3±1.43	5.1±1.32	4.5±1.10	4.01±1.32	5.2±1.19	5.0±0.98	4.3±1.29	3.9±0.60
Control (Placebo)					5.9±1.14	8.6±0.79	6.0±1.10	5.8±1.33
Standard (5 mg/kg glibenclamide)					4.6±1.60	3.7±1.34	3.1±1.09	2.7±1.43

Data are expressed as mean ± SD (n=3). *****p = 0.05 indicates "statistically significant" compared to control. HE: *n*-hexane extract, DCME: dichloromethane extract, EAE: ethyl acetate extract, ME: methanol extract.

In vitro membrane stabilizing activity revealed that the dichloromethane, ethyl acetate and methanol extracts of seed showed higher percentage (%) inhibition of hypotonic solution-induced hemolysis as compared to the standard acetyl salicylic acid (Table 2). So, it can be proposed that the inhibition of erythrocyte lysis property of *A. sowa* could be the possible mechanism for its anti-inflammatory activity.

To find out the preliminary hypoglycemic activity, all extracts were subjected to assay on Swiss-albino mice model. The dichloromethane and ethyl acetate extracts of stem and seed displayed moderate blood glucose lowering effect (Tables 3 and 4).

CONCLUSION

The seed and stem extracts of *A. sowa* were found to be very effective in membrane stabilization of RBCs. Further extensive studies are needed to isolate the bioactive compounds and to investigate the underlying mechanisms for these bioactivities.

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