

Chemical and Biological Investigations of Leaves of *Abroma augusta* Linn.

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Abroma augusta Linn. (Family- Sterculiaceae), commonly known as Ulatkambal in Bengali and Hindi, is an evergreen tree widely distributed (native or cultivated) throughout the warm area of India such as Uttar Pradesh, Sikkim, Khasi Hills, Assam and in Bangladesh throughout the hilly regions of Sylhet. It is also found in Java, Philippines and China (Ahmad *et al.*, 2003). *A. augusta* has a long history of medicinal uses in the Ayurvedic system. It regulates the menstrual flow and also used as abortifacient and anti-fertility agents. In India, it is used in dysmenorrhea but in Indonesia it is used as anti-scabies, anti-dermatitis, anti-inflammatory and analgesic agents. The leaves and stems of *A. augusta* are used by the traditional healers of Bogra district of Bangladesh, but the roots are used in Jessore district (Hossain *et al.*, 2010). The plant is being used as a uterine tonic and to treat amenorrhoea. Leaves are useful in the treatment of uterine disorder, diabetes, rheumatic pains and headache with sinusitis. Leaves and stem are used as demulcent. An infusion of fresh leaves and stems in cold water is very efficacious in gonorrhoea. The root-bark is used as emmenagogue and uterine tonic (Gupta *et al.*, 2011; Das *et al.*, 2012).

Literature survey has revealed a number of chemical and biological investigations of *A. augusta* (Halim *et al.*, 2001; Laizumanet *et al.*, 2010; Sutapa *et al.*, 2012; Uddin *et al.*, 2012; Nahar *et al.*, 1994; Jayaprakasam *et al.*, 2015). As a part of our continuing investigations with medicinal plants of Bangladesh (Jahan *et al.*, 2010), the present study was undertaken to comprehensively conduct the chemical and biological investigations of leaves of *A. augusta*. We, herein, report beta-sitosterol, stigmasterol and lupeol from the

leaf extract. The antioxidant, cytotoxic, thrombolytic and membrane stabilizing activities of the leaves are also reported here.

¹H-NMR spectra were recorded using a Bruker AMX-500 (500 MHz) instrument and the spectra were referenced to the residual non deuterated solvent signal. PTLC (20 × 20 cm) and TLC (20 × 5 cm) were carried out using Merck Si gel PF₂₅₄ on glass plate at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized by spraying the developed plates with vanillin-sulfuric acid solution (1:100) followed by heating for 5 minutes at 110°C. All solvents used in this study were of reagent grade. The standard compounds were collected from the Phytochemical Research Laboratory of the Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh. The reference standards for the biological investigations were collected from Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh.

The leaves of *A. augusta* were collected from Sylhet, Bangladesh. The plant was identified at Bangladesh National Herbarium, where a voucher specimen has been deposited for this collection (DACB accession number: 37591). The leaves were dried under sun light for several days and then oven dried for 24 hours at considerably low temperature (not more than 40°C) for better grinding.

The leaf-powder (1200 gm) was taken in a clean, amber color reagent bottle (5 liters) and soaked in 3.0 L of methanol. The container with its content was sealed by bottle cap and kept for 10 days accompanying occasional shaking and stirring. The mixture was then filtered through a fresh cotton plug and finally with

Whatman Number 1 filter paper. The filtrate was allowed to dry to yield the crude extract. Solvent-solvent partitioning was done using the modified Kupchan method (Van Wagenen *et al.*, 1993). A portion of the crude extract (5 gm) was dissolved in 10% aqueous methanol and extracted with petroleum ether, then with carbon tetrachloride followed by chloroform and finally with water to provide petroleum ether soluble fraction (PESF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF), respectively. Later on, the fractions were allowed to concentrate.

The CTSF was subjected to purification by column chromatography packed with silica gel (Kieselgel 60, mesh 70-230). Slurry of silica gel in petroleum ether was added into a glass column having the length and diameter of 55 cm and 1.1 cm, respectively. The column was then eluted with petroleum ether, followed by mixtures of petroleum ether and ethyl acetate of increasing polarity, then by ethyl acetate and finally with ethyl acetate and methanol mixtures of increasing polarity. Total 184 fractions (about 20 ml each) were collected.

β -sitosterol (3.0 mg) was obtained as white amorphous powder through preparative TLC using solvent system toluene and ethyl acetate (99:1) of column fractions 71-77 whereas stigmasterol (2.6 mg) was isolated from column fractions 81-83 as needle shaped crystalline mass. On the other hand, preparative TLC of the fractions 56-58 using mixture of toluene and ethyl acetate (98:2) yielded lupeol (2.7 mg).

β -sitosterol: whitish amorphous powder; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 5.35 (1H, m, J = 6.0 Hz, H-6), 3.52 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.93 (3H, d, J = 6.5 Hz, Me-21), 0.85 (3H, t, J = 7.8 Hz, Me-29), 0.83 (3H, d, J = 7.5 Hz, Me-26), 0.82 (3H, d, J = 7.5 Hz, Me-27), 0.69 (3H, s, Me-18).

Stigmasterol: colorless needle shaped crystals; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 5.34 (1H, m, Hz, H-6), 5.15 (1H, dd, J = 6.5, 15.0 Hz, H-22), 5.02 (1H, dd, J = 9.0, 15.0 Hz, H-23), 3.51 (1H, m, H-3 α), 1.00 (3H, br. s, Me-19), 0.91 (3H, d, J = 6.5 Hz, Me-21), 0.83 (3H, d, J = 7.0 Hz, Me-26), 0.81 (3H, d, J = 4.5 Hz, Me-27), 0.80 (3H, t, J = 7.8 Hz, Me-29), 0.67 (3H, br. s, Me-18).

Lupeol: $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 4.70 (1H, br. s, H_a-29), 4.65 (1H, br. s, H_b-29), 3.20 (1H, m, H-3), 2.12 (1H, m, H-19), 1.67 (3H, s, H₃-30), 1.02 (3H, s, H₃-26), 0.96 (3H, s, H₃-23), 0.92 (3H, s, H₃-27), 0.87 (3H, s, H₃-25), 0.81 (3H, s, H₃-28), 0.79 (3H, s, H₃-24).

Total phenolic content of extractives leaves of *A. augusta* was measured employing the method of Skerget *et al.*, (2005) involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard (Majhenic *et al.*, 2007).

Following the method of Meyer *et al.*, (1982) the cytotoxic properties of the extractives of the leaves of *A. augusta* was determined against *Artemia salinain* an one-day *in vivo* assay. Vincristine sulfate was used as positive control.

The thrombolytic activity of the crude extract and partitionates was assessed by the method developed by Prasad *et al.*, (2007).

According to the method developed by Shinde *et al.* (1999) and modified method by Sikder *et al.* (2011), the membrane stabilizing activity of the extractives was determined by evaluating their ability to prevent hypotonic solution- and heat-induced hemolysis of human erythrocyte.

Chromatographic separation and purification of soluble fraction of methanolic extract of *A. augusta* led to the isolation of two phytosterols identified as β -sitosterol and a stigmasterol and a triterpene- lupeol which were characterized by analysis of $^1\text{H-NMR}$ spectral data and comparison with reported values. The identity of each compound was further substantiated by co-TLC with authentic samples.

The methanol extract and its different fractions were investigated to determine total phenolic content by Folin-Ciocalteu reagent, cytotoxicity by brine shrimp lethality, thrombolytic and membrane stabilizing activities.

The amount of total phenolic content differed in different extractives and ranged from 50.58 - 266.38 mg of gallic acid equivalent (GAE)/gm of extractives. Among all extractives of leaves of *A. augusta*, the highest phenolic content was found in chloroform soluble fraction (266.38 mg of GAE/gm of extractives) which is followed by petroleum ether soluble fraction (251.81 mg of GAE/gm of extractives) (Table 1).

In brine shrimp lethality bioassay, vincristine sulfate (VS) was used as positive control and the LC₅₀ was found to be 0.45 µg/ml. Among the extractives, the chloroform soluble fraction demonstrated most significant cytotoxic activity as evident from the LC₅₀ value of 2.69 µg/ml (Table 1).

Table 1. Total phenolic content, cytotoxicity, thrombolytic and membrane stabilizing activities of leaves of *A. augusta*.

Sample	Total phenolic content (mg of GAE/ gm of dried extract)	Brine shrimp lethality bioassay LC ₅₀ (µg/ml)	Thrombolytic activity (in terms of % of clot lysis)	Membrane stabilizing activity	
				% inhibition of haemolysis (hypotonic solution-induced)	% inhibition of haemolysis (heat-induced)
VS	-	0.45	-	-	-
ME	86.63	6.86	32.87	76.43	45.36
PESF	251.81	10.09	32.39	85.83	51.18
CTSF	183.30	3.20	44.90	66.45	22.45
CSF	266.38	2.69	11.23	87.50	10.71
AQSF	50.58	15.83	41.51	95.22	23.44
Blank	-	-	10.27	-	-
Streptokinase	-	-	66.77	-	-
ASA	-	-	-	71.91	42.20

ME= Methanol crude extract; PESF= Petroleum ether soluble fraction; CTSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction and AQSF= Aqueous soluble fraction; BHT= Butylated hydroxytoluene; VS= Vincristine sulfate; ASA= Acetylsalicylic acid.

In order to discover cardio protective drugs from natural sources, the crude extract and its different fractions were assessed for thrombolytic activity and the results are presented in table 1. Addition of 100 µl streptokinase, a positive control (30,000 I.U.), to the clots demonstrated 66.77% lysis of clot. On the other hand, distilled water was applied as negative control which exhibited 10.27% lysis of clot (Table 1).

In hypotonic solution-induced condition the aqueous soluble fraction at 1.0 mg/ml inhibited highest percentage of haemolysis of RBC (95.22%) while acetylsalicylic acid (0.10 mg/ml) showed 71.91% inhibition. On the other hand, at heat-induced condition the petroleum ether soluble fraction at 1.0 mg/ml inhibited highest percentage of haemolysis of RBC (51.18%) as compared to the standard acetyl salicylic acid (0.10 mg/ml) displayed by 42.20% inhibition.

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