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Original Article

Investigation of biological activities of *Allamanda blanchetii*, the violet Allamanda

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ABSTRACT

Objectives: The objective of the study was to evaluate *Allamanda blanchetii* extractives for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities.
Methods: The plant extractives were evaluated for their phenolic content and their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Brine shrimp lethality bioassay was conducted to identify cytotoxic potential of the extractives. The test samples were also involved in thrombolytic and membrane stabilizing activity assays to evaluate their abilities to promote clot lysis and to stabilize erythrocyte membrane under hypotonic and heat induced conditions. The extractives were involved in disc diffusion assay to measure their ability to give zones of inhibition in cultured bacterial medium.
Results: In DPPH free radical scavenging assay, the carbon tetrachloride soluble fraction demonstrated the highest free radical scavenging activity ($IC_{50} = 40.50 \pm 0.32$ μg/ml) where as, in brine shrimp lethality bioassay, the hexane soluble fraction revealed the highest cytotoxic activity with LC_{50} value of 0.78 ± 0.74 μg/ml. While evaluating the thrombolytic activity of the extractives, the chloroform soluble fraction showed $32.50 \pm 0.63\%$ of clot lysis. This fraction at 1.0 mg/ml concentration also inhibited $46.74 \pm 0.73\%$ and $41.33 \pm 0.59\%$ of haemolysis of RBCs induced by hypotonic solution and heat as compared to 71.90% and 42.12% by acetyl salicylic acid (0.10 mg/ml), respectively. In disc diffusion assay, the extractives of *A. blanchetii* revealed zone of inhibition ranging from 7.0 to 13.0 mm.
Conclusion: Further chemical and bioassay guided investigation on violet Allamanda must be conducted for isolation, identification and characterization of the bioactive constituents.

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1. Introduction

Allamanda blanchetii A. DC. (Synonym: *Allamanda violacea* Gardn.), commonly known as purple Allamanda, is an ornamental plant of *Allamanda* genus in the Apocynaceae family. All parts of the plant are poisonous if ingested. *A. blanchetii* is commonly used as an ornamental plant. The compounds plumericin, isoplumericin and 5,6-dimethoxycoumarin (unckalin) were previously isolated from *A. blanchetii*.[1] Many active phytochemicals have been isolated from the roots as well.[2]

As part of our ongoing investigations on medicinal plants of Bangladesh, the crude methanol extract of leaves of *A. blanchetii* growing in Bangladesh as well as its organic and aqueous soluble fractions were studied for the antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities for the first time and we, here in, report the results of our preliminary investigations.

2. Materials and methods

2.1. Plant materials

The leaves of *A. blanchetii* were collected from Dhaka, Bangladesh, in May 2012. A voucher specimen (DUSH – 10772) for this plant has been maintained in Dhaka University Salar Khan Herbarium for future reference.

The sun dried and powdered leaves (500 g) were macerated in 1.5 L of methanol for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of the concentrated methanol extract was fractionated by modified Kupchan partition protocol[3] and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF, 1.5 g), carbon tetrachloride (CTCSF, 1.5 g), chloroform (CSF, 1 g) and aqueous (AQSF, 0.5 g) soluble materials. The residues were then stored in the refrigerator until further use.

2.1.1. Total phenolic content

The total phenolic content of the extractives was determined with Folin–Ciocalteu reagent by using the method developed by Harbertson and Spayd (2006).[4]

Table 2 – Thrombolytic activity of *A. blanchetii*.

Samples/standard	% of lysis of RBCs
ME	30.95 ± 0.56
HXSF	23.68 ± 0.34
CTCSF	23.10 ± 0.11
CSF	32.50 ± 0.63
AQSF	29.35 ± 0.49
Water	3.79 ± 0.55
SK	66.77 ± 0.36

ME = Methanolic crude extract; HXSF = Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; SK = Streptokinase.

2.1.2. DPPH free radical scavenging assay

Following the method developed by Brand-Williams et al (1995),[5] the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

2.1.3. Phosphomolybdenum antioxidant assay

The total antioxidant capacity of the extractives was evaluated by the phosphomolybdenum assay method.[6]

2.1.4. Brine shrimp lethality bioassay

This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a single day in vivo assay.[7] Vincristine sulphate was used as positive control.

2.1.5. Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad et al (2006)[8] by using streptokinase (SK) as positive control.

2.1.6. Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale et al (2008).[9]

Table 1 – Total phenolic content, free radical scavenging activity, phosphomolybdenum total antioxidant capacity, and cytotoxic activity of *A. blanchetii*.

Samples/standards	Total phenolic content (mg of GAE/g of dried extract)	Free radical scavenging activity IC_{50} (μg/ml)	Total antioxidant capacity	Brine shrimp lethality bioassay LC_{50} (μg/ml)
ME	10.89 ± 0.09	49.34 ± 0.61	0.83 ± 0.52	14.04 ± 0.45
HXSF	10.77 ± 0.21	58.54 ± 0.52	0.75 ± 0.64	0.78 ± 0.74
CTCSF	21.08 ± 0.41	40.50 ± 0.32	1.58 ± 0.32	56.12 ± 0.22
CSF	1.39 ± 0.77	83.20 ± 0.57	0.61 ± 0.57	32.87 ± 0.70
AQSF	0.28 ± 0.12	119.21 ± 0.43	0.29 ± 0.77	92.82 ± 0.19
VS	–	–	–	0.45 ± 0.04
BHT	–	27.50 ± 0.54	–	–
Ascorbic acid	–	5.80 ± 0.21	–	–

ME = Methanolic crude extract; HXSF = Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; BHT = Butylated hydroxytoluene; VS = Vincristine sulphate.