academicJournals

Vol. 8(1), pp. 63-67, 3 January, 2014
DOI: 10.5897/JMPR12.1273
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antioxidant, total phenolic, membrane stabilizing and antimicrobial activity of *Allamanda cathartica* L.: A medicinal plant of Bangladesh

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Accepted 19 July, 2013

The methanol extract of the leaf of *Allamanda cathartica* L. as well as its hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screening for antioxidant, membrane stabilizing and antimicrobial activities. The antioxidant potential was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteau reagents using butylated hydroxytolune (BHT) and ascorbic acid as standards. The carbon tetrachloride soluble fraction revealed the highest free radical scavenging activity (IC_{50} = 47.5±0.11 µg/ml) which could be correlated to its total phenolic content of 59.31±0.47 mg of gallic acid equivalent (GAE)/g of extractives. In hypotonic solution and heat induced conditions, the aqueous soluble fraction inhibited haemolysis of human erythrocyte by 69.49±0.49 and 40.0±0.75%, respectively. Here, acetyl salicylic acid (0.1 mg/ml) was used as reference showing 72.79 and 42.12% of haemolysis of red blood cells (RBCs) in hypotonic solution and heat induced conditions, respectively. The carbon tetrachloride soluble fraction of *A. cathartica* demonstrated activity against microbial growth with zone of inhibition ranging from 5.0 to 8.5 mm. This fraction demonstrated 8.5 mm zone of inhibition against *Bacillus megaterium*.

Key words: *Allamanda cathartica*, total phenolic content, 1,1-diphenyl-2-picrylhydrazyl (DPPH), free radical scavenging activity, membrane stabilizing activity, zone of inhibition.

INTRODUCTION

Allamanda cathartica L. (Synonyms: *Echites verticillata* Sessé & Moç, *Orelia grandiflora* Aublet, *Allamanda grandiflora* (Aublet) Poiret in Lam, *Allamanda hendersonii* W. Bull ex Dombrain.; Bengali name: Ghanta phul) commonly known as Golden Trumpet, Yellow Bell or Buttercup Flower, is a perennial shrub that can grow up to a height of 15 feet tall or more. The plant is native to Brazil, but widely cultivated throughout the tropics. *A. cathartica* is primarily used as an ornamental plant. The plant is used to relieve coughs and to clear the nasal passages. The leaves are also made into decoctions for use as a purgative. This plant has anti-bacterial and anti-cancer properties. It is also widely used in the treatment of jaundice. The root and stem of this plant contain two rare lactones which are active against polio virus and pathogenic fungi. Root is also used in various formulations to treat malarial symptoms. Sap is used to eliminate intestinal worms. The plant is also used as laxative and emetic (David, 1997). The leaves stem and branches of this plant are used against snake bite (Gomes et al., 2010).

As part of our ongoing investigations on medicinal plants of Bangladesh (Kaisar et al., 2011; Sharmin et al., 2012), the methanol extract of leaves of *A. cathartica* as well as its organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total

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Table 1. Total phenolic content and free radical scavenging activity of *A. cathartica*.

Samples/Standards	Total phenolic content (mg of GAE/g of extractives)	DPPH free radical scavenging activity (IC_{50} µg/ml)
ME	30.56±0.81	167.40±0.59
HXSF	22.43±1.24	181.93±1.21
CTCSF	59.31±0.47	47.5±0.11
CSF	0.375±0.39	419.87±0.34
AQSF	20.56±0.24	351.85±0.22
BHT	-	27.5±0.54
Ascorbic acid	-	5.8±0.21

BHT: Butylated hydroxytolune; ME: methanolic crude extract; HXSF: hexane soluble fraction; CTCSF: carbon tetrachloride soluble fraction; CSF: chloroform soluble fraction; AQSF: aqueous soluble fraction.

phenolic content and free radical scavenging property, membrane stabilizing and antimicrobial activities for the first time and were reported, the results of our preliminary investigations.

MATERIALS AND METHODS

Collection of plant and extraction

The leaves of *A. cathartica* were collected at their fully mature form in April 2011 from Mirpur Botanical Garden and a voucher specimen (DACB – 36081) has been deposited in Bangladesh National Herbarium for future reference.

The collected plant materials were cleaned, sun dried and pulverized. The powdered plant material (700 g) was soaked in 2.0 L of methanol at room temperature 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 (van Wagenen et al., 1993) partition protocol and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF, 1.0 g), carbon tetrachloride (CTCSF, 1.2 g), chloroform (CSF, 1.5 g) and aqueous (AQSF, 1.0 g) soluble materials. The residues were then stored in a refrigerator until further use.

Total phenolic content

The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006).

DPPH free radical scavenging assay

Following the method developed by Brand-Williams et al. (1995), 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.

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).

Antimicrobial screening

Antimicrobial activity was determined by disc diffusion method (Bayer et al., 1966).

Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the antioxidant potential in terms of total phenolic content and free radical scavenging property, membrane stabilizing and antimicrobial activities of different organic and aqueous soluble materials of the methanol extract of *A. cathartica* leaves.

In DPPH free radical scavenging activity assay, all the fractions demonstrated mild to moderate free radical scavenging potential with IC_{50} values ranging from 47.5 to 419.87 µg/ml. The highest free radical scavenging activity was demonstrated by the carbon tetrachloride soluble fraction (IC_{50} = 47.5±0.11 µg/ml) which could be correlated to its phenolic content 59.31±0.47 mg of GAE/g of extractives (Table 1, Figures 1 and 2).

The membrane stabilizing activity of *A. cathartica* was also determined. All the extractives significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat induced conditions, as compared to the standard acetyl salicylic acid. In hypotonic solution and heat induced conditions, the aqueous soluble fraction inhibited 69.49±0.49 and 40.00±0.75% haemolysis of RBCs, respectively as compared to 72.79 and 42.12% inhibition by acetyl salicylic acid (0.10 mg/ml), respectively (Table 2 and Figure 3).

Table 2. Effect of different extractives of leaf of *A. cathartica* on hypotonic solution and heat induced haemolysis of erythrocyte membrane.

Sample	Inhibition of haemolysis (%)	
	Hypotonic solution induced	Heat induced
ME	44.62±0.26	37.7±0.18
HXSF	2.04±0.33	17.4±0.66
CTCSF	46.26±0.01	39.4±0.49
AQSF	69.49±0.49	40.0±0.75
ASA	72.79±0.47	42.12±-0.38

ME: Methanolic crude extract; HXSF: hexane soluble fraction; CTCSF: carbon tetrachloride soluble fraction; AQSF: aqueous soluble fraction; ASA: acetyl salicylic acid.

Table 3. Antimicrobial activity of *A. cathartica*.

Test microorganism	Diameter of zone of inhibition (mm)	
	CTCSF	Ciprofloxacin
Bacillus cereus	10.0±0.43	45.0±2.01
Bacillus megaterium	11.5±0.28	42.0±1.17
Bacillus subtilis	12.0±0.62	42.0±0.73
Sarcina lutea	-	42.0±0.23
Staphylococcus aureus	-	42.0±0.56
Escherichia coli	-	42.0±0.43
Pseudomonas aeruginosa	-	42.0±1.11
Salmonella typhi	13.0±0.13	45.0±0.73
Salmonella paratyphi	11±0.38	47.0±2.33
Shigella boydii	-	34.0±0.58
Shigella dysenteriae	-	42.0±0.22
Vibrio mimicus	-	40.0±0.45
Vibrio parahaemolyticus	-	35.0±0.44
Candida albicans	-	38.0±0.49
Aspergillus niger	-	37.0±0.33
Sacharomyces cerevacae	-	38.0±0.11

CTCSF: Carbon tetrachloride soluble fraction.

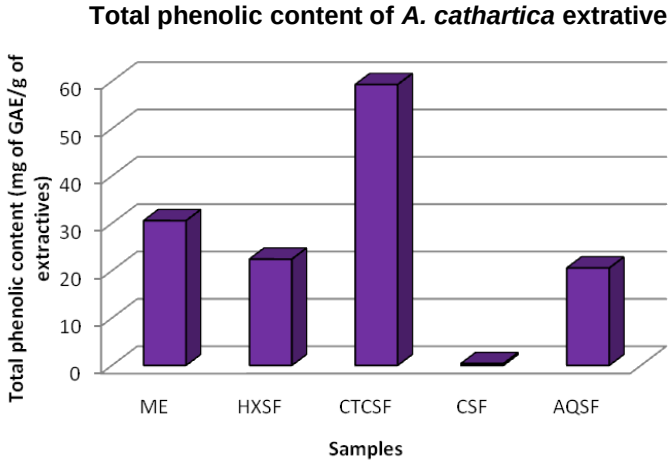

Figure 1. Free radical scavenging activity of *A. cathartica.*

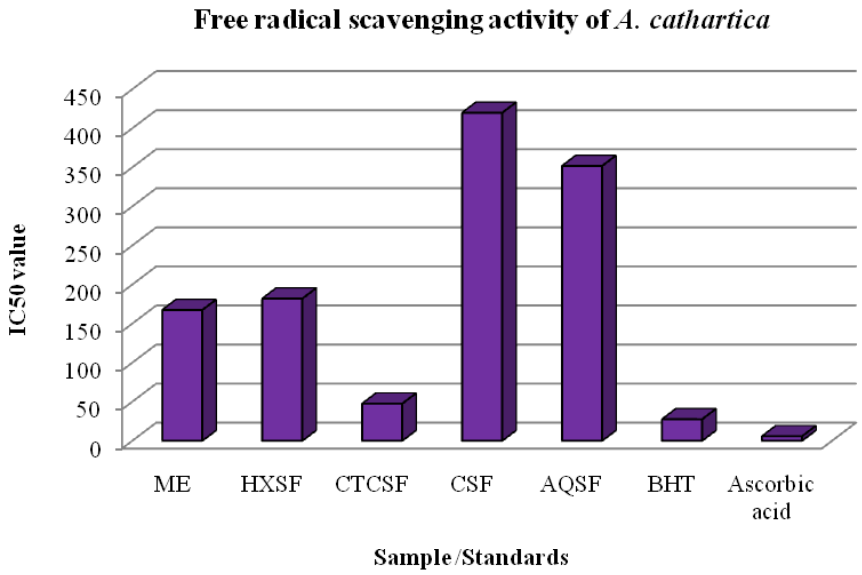

Figure 2. Free radical scavenging activity of *A. cathartica.*

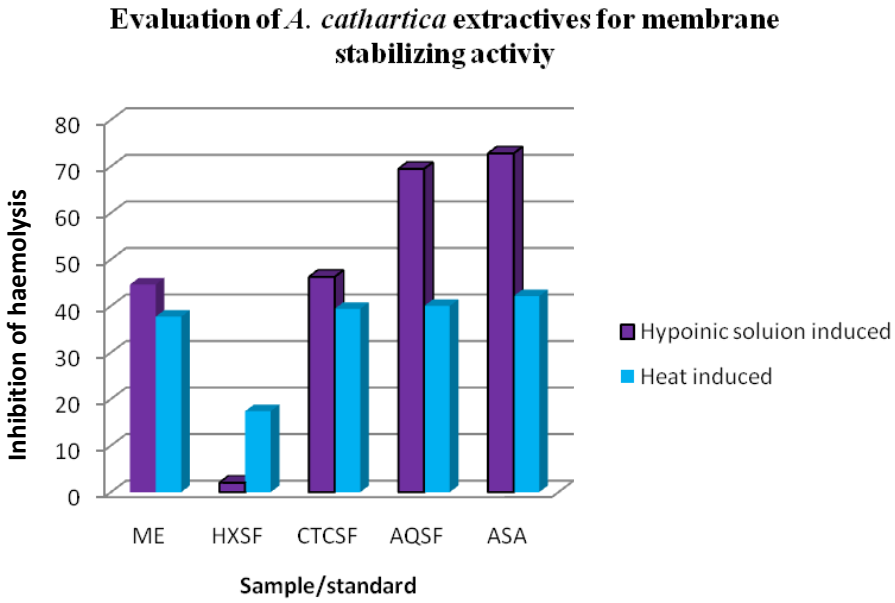

Figure 3. Effect of different extractives of leaf of *A. cathartica* on hypotonic solution and heat induced haemolysis of erythrocyte membrane.

The antimicrobial activity of *A. cathartica* test samples was evaluated against five Gram positive and eight Gram negative bacteria and three fungi and the results were compared with standard antibiotic, Ciprofloxacin. The carbon tetrachloride soluble fraction displayed zone of inhibition ranging from 5.0 to 8.5 mm. This fraction revealed 8.5 mm zone of inhibition against *Bacillus megaterium* (Table 3).

It is clearly evident from the aforementioned findings that the test samples of *A. cathartica* have significant membrane stabilizing activity, mild to moderate antioxidant and weak antimicrobial potentials. Therefore, the plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

ACKNOWLEDGEMENT

The authors wish to acknowledge the phytochemical research laboratory of State University of Bangladesh.

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