Bangladesh Pharmaceutical Journal ***17****(1): 99-101, 2014*

In vitro Antioxidant, Cytotoxic and Membrane Stabilizing Activities of *Bauhinia acuminata* L.

Mohammad Firoz Khan[1], Rabeya Islam Shilpi[1], Ridwan Bin Rashid[2] and Mohammad A. Rashid[3]

[1]Department of Pharmacy, State University of Bangladesh, Dhaka- 1205, Bangladesh
[2]Department of Microbiology, University of Dhaka, Dhaka- 1000, Bangladesh
[3]Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka- 1000, Bangladesh

Abstract

The crude methanol extract of leaves of *Bauhinia acuminata* and its Kupchan fractions were screened for antioxidant, cytotoxic and membrane stabilizing activities. Among all partitionates the aqueous soluble fraction of *B. acuminata* demonstrated the highest antioxidant activity with IC_{50} value of 7.22±0.200 µg/ml. Moreover, the carbon tetrachloride soluble fraction showed significant cytotoxic activity having LC_{50} value of 12.13±0.215 µg/ml. On the other hand, in hypotonic solution- and heat- induced conditions, the crude methanol extract inhibited haemolysis of human erythrocyte by 63.94±0.14% and 51.95±0.20%, respectively as compared to 81.97±0.77% and 42.11±0.39% demonstrated by the standard acetyl salicylic acid.

Key words: *Bauhinia acuminata*, antioxidant, cytotoxic and membrane stabilizing.

Introduction

Bauhinia acuminata L. (Common Name- Dwarf White Bauhinia, Family- Fabaceae) is a species of flowering shrub native to tropical southeastern Asia. The bark, flower and root of the *B. acuminata* are used for various skin diseases, worms, tumours and diabetes (Ayurvedic Medicinal Plants). The bark and leaves of *B. acuminata* is used to treat biliousness (Timothy, 1999), a remedy recommended by the Indian Vaiydas (Khare, 2007). In Malaysia and Indonesia the plant is used in the treatment of common cold and cough (Timothy, 1999). While in India the leaves and bark of this plant are used for treating asthma attack (Khare, 2007). Moreover, the leaf of *B. acuminata* is used to treat bladder stone, venereal diseases, leprosy, asthma and digestive diseases (FloraCafe). In contribution of our ongoing efforts to study medicinal plants of Bangladesh (Sikder *et al*., 2013, Chowdhury *et al.*, 2013, Sharmin *et al.*, 2013), the present study has been undertaken and we, herein, report the antioxidant, cytotoxic and membrane stabilizing properties of the leaf of *B. acuminata* for the first time.

Materials and Methods

Plant materials: The leaves of *B. acuminata* were collected from Khulna and a voucher specimen (DUSH-10775) for this plant sample has been deposited in the Department of Botany, University of Dhaka for future reference.

Extraction and fractionation: The collected palnt parts were sun dried for several days and then oven dried for 24 hours at 40° C to facilitate grinding. The powdered whole plant (600 gm) of *B. acuminata* was extracted with about 1.5 L methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40-45° C) and pressure. The concetrated methanol extract (ME) was partitionated by the modified Kupchan method (Van Wagenen *et al*., 1993) and the resultant patitionates i.e., methanol extract (ME), pet ether (PE), carbon tetrachloride (CT), chloroform (CL) and aqueous (AQ) soluble materials were used for different biological screenings.

Correspondence to: Mohammad A. Rashid; Tel.: 880-2-9661900, Extn. 8137; Fax: 880-2-8615583; E-mail: rashidma@du.ac.bd

Antioxidant activity: The free radical scavenging activity of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method of Brand –Williams (Wichi, 1988; Auddy *et al*., 2003). Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated by using *tert*- butyl-1-hydroxytoluene (BHT), a potential antioxidant as positive control.

Cytotoxic screening: This technique was applied for the determination of general toxic property of the plant extractives by using established protocol (Meyer *et al.*, 1982; McLaughlin *et al*., 1998) against *Artemia salina* in a 1-day *in vivo* assay. Vincristine sulphate was used as positive control.

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).

Statistical Analysis: Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD.

Result and Discussion

The methanol extract of *B. acuminata* as well as different Kupchan partitionates derived from it were subjected to assay for total phenolic content, free radical scavenging activity, cytotoxic and membrane stabilizing activities. The total phenolic content in crude methanol extract and its pet ether, carbon tetrachloride, chloroform and aqueous soluble fractions were found to be 48.06±0.50, 47.81±0.63, 13.98±0.41, 18.43±0.07 and 18.55±0.10 mg GAE/gm of sample, respectively (Table 1). The result indicated the presence of highest amount of phenolic compounds in the crude methanol extract and its pet ether soluble fraction.

Different partitionates of methanol extract of *B. acuminata* were also tested for free radical scavenging activity using DPPH. The IC_{50} values of the extractives were found in the range of 7.22±0.200 µg/ml to 79.93±0.412 µg/ml. Among all the partitionates the aqueous soluble fraction and crude methanol extract showed highest (IC_{50} = 7.22±0.200 µg/ml and 8.82±0.412 µg/ml) free radical scavenging activity (Table 1).

Table 1. IC_{50} values of standard and different partitionates of *B. acuminata* in DPPH assay.

Samples	IC_{50} (µg/ml)
BHT	21.20 ± 0.520
AQ	7.22 ± 0.200
ME	8.82 ± 0.412
CL	16.03 ± 0.312
CT	26.88 ± 0.215
PE	79.93 ± 0.412

BHT= tert- butyl-1-hydroxytoluene, AQ= Aqueous soluble fraction, ME= Methanol extract, CL= Chloroform soluble fraction, CT= Carbon tetrachloride soluble fraction, PE= Pet ether soluble fraction.

In Brine shrimp lethality bioassay the median lethal concentration (LC_{50}) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration and the best-fit line was obtained from the graph by means of regression analysis. Among all the partitionates of crude methanol extract of *B. acuminata*, the carbon tetrachloride soluble fraction displayed highest lethality having LC_{50} value 12.13±0.215 µg/ml.

Table 2. LC_{50} values of standard and different partitionates of *B. acuminata* in brine shrimp lethality bioassay.

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 30.8x + 60.64	0.972	0.451±0.041
PE	y = 47.71x - 14.52	0.866	22.51±0.122
CT	y = 36.64x + 10.29	0.926	12.13±0.215
CL	y = 34.62x - 9.196	0.911	51.27±0.145
ME	y = 44.89x - 17.00	0.881	31.08±0.035

VS=Vincristine sulfate, PE= Pet ether soluble fraction, CT=Carbon tetrachloride soluble fraction, CL= Chloroform soluble fraction, ME= Methanol extract.

At concentration of 1.0 mg/ml, different partitionate fractions of *B. acuminate* protected the haemolysis of RBC induced by hypotonic solution and heat as compared to the standard acetyl salicylic acid. The crude methanol extract inhibited 63.94±0.14% and 51.95±0.20% of haemolysis of RBC induced by hypotonic solution and

heat as compared to 81.97±0.77% and 42.11±0.39% by acetyl salicylic acid, respectively (Table 3).

Table 3. Percentage (%) inhibition of heat and hypotonic solution induced haemolysis of erythrocyte membrane by standard and different partitionates of *B. acuminate*.

Samples	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	-	-
CL	31.20 ± 0.03	64.03 ± 0.51
ME	51.95 ± 0.20	63.94 ± 0.14
CT	84.35 ± 0.15	38.76 ± 0.52
PE	38.18 ± 0.86	29.34 ± 1.01
ASA	42.11 ± 0.39	81.97 ± 0.77

CL= Chloroform soluble fraction, ME= Methanol extract, CT= Carbon tetrachloride soluble fraction, PE= Pet ether soluble fraction, ASA= Acetyl salicylic acid

Acknowledgement

The authors wish to acknowledge the Phytochemical Research Laboratory of State University of Bangladesh for providing necessary facilities to carry out the work.

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