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Bioactivities of *Artocarpus chaplasha* Roxb. and *Bougainvillea spectabillis* Willd.

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Abstract

The methanol extracts of leaf of *Artocarpus chaplasha* Roxb. and *Bougainvillea spectabillis* Willd. as well as their petroleum ether, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. The antioxidant potential was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteau reagent using butylated hydroxytolune (BHT) and ascorbic acid as standards. The aqueous soluble fraction of the crude methanol extract of *A. chaplasha* and the methanolic crude extract of *B. spectabillis* demonstrated the highest free radical scavenging activity with the IC_{50} of 15.60±0.27 µg/ml and 18.33±0.17 µg/ml, which could be correlated to their total phenolic content of 61.26±0.23 and 50.00±0.19 mg of GAE/gm of extractive, respectively. Different extractives of *A. chaplasha* and *B. spectabillis* showed clot lysis activity ranging from 2.56±0.68% to 17.07±0.23% and 2.24±0.71% to 17.59±0.74% as compared to 66.77% and 3.791 % produced by the standard streptokinase and water, respectively. In hypotonic solution and heat induced conditions, the crude extract of *A. chaplasha* inhibited the haemolysis of human erythrocyte by 41.96±0.87% and 40.00±0.78%, while the carbon tetrachloride soluble fraction of methanol extract of *B. spectabillis* inhibited haemolysis by 65.68±0.36% and 35.62±0.82%, respectively. Here, acetyl salicylic acid (0.1 mg/ml) was used as reference showing 72.79% and 42.12% of haemolysis of RBCs in hypotonic and heat induced conditions, respectively. Among the extractives of both plants, the crude methanol extract of *A. chaplasha* and the chloroform soluble fraction of *B. spectabillis* extract revealed mild to moderate antimicrobial activity with zone of inhibition ranging from with 7.0 to 13.0 mm. The general toxicity was determined by brine shrimp lethality bioassay where the pet-ether soluble fraction of *A. chaplasha* (LC_{50} 0.781±0.36 µg/ml) and the aqueous soluble partitionate of *B. spectabillis* (LC_{50} 1.28±0.57 µg/ml) suggested the presence of considerable bioactive principles.

Key words: *Artocarpus chaplasha*, *Bougainvillea spectabillis*, antioxidant, DPPH, thrombolytic, membrane stabilizing, antimicrobial, cytotoxicity.

Introduction

Natural products have contributed a lot for developing new drug molecules. Until today, medicinal chemists look around plants, herbs, microorganisms, marine resources etc. to incorporate new lead compounds. Bangladesh is blessed with numerous medicinal plants used for traditional healing (Butler, 2005; Gan *et al.,* 2010). Very few of them have been scientifically evaluated for exploring their chemical constituents and biological activities. In the process of our continuous study with medicinal plants of Bangladesh (Sikder *et al.*, 2011; Kaisar *et al.*, 2011), we have investigated *Artocarpus chaplasha* Roxb. and *Bougainvillea spectabillis* Willd. in the current study.

A. chaplasha Roxb. (Synonyms: *A. chama* Buch.-Ham., *Urostigma chrysophthalmum* Miq.; Bengali name: Chapalish) belongs to the family Moraceae. The plant is native to northeastern India, lower Myanmar and the Andaman and Nicobar Islands. It is also available in sub-Himalayan tract, Nepal, and other parts of East Asia (Flora of China, 2012).

B. spectabillis Willd. (Synonyms: *B. bracteata* Pers., *B. brasiliensis* Raeusch; Bengali name: Bagan bilash) belongs to the family Nyctaginaceae. It is

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native to South America and is considered as a popular ornamental plant in the Philippines. In Bangladesh, the plant is widely distributed. The root of the plant possesses hypoglycaemic (Jawla, 2011) and anti-hyperlipidemic properties (Saikia and Lama, 2011)

In this investigation, the methanol extracts of leaves of *A. chaplasha* and *B. spectabillis* growing in Bangladesh as well as their organic and aqueous soluble fractions were studied for assessing the antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities and, we herein, report the results of our preliminary studies for the first time.

Materials and Methods

Collection of plant materials and extraction: The leaves of *A. chaplasha* were collected in March 2012 from Mirpur Botanical Garden, Dhaka and a voucher specimen (DACB-37786) for this collection has been deposited in Bangladesh National Herberium. On the other hand, the leaves of *B. spectabillis* were collected in April 2012 from Baldha Garden, Dhaka and a voucher specimen (DUSH-3630) has been deposited in Salar Khan Herbarium, Department of Botany, and University of Dhaka.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 g each) of both the plants were separately soaked in 2.0 liter of methanol at room temperature for 7 days. The extracts were 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 each of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method (Van Wagenen *et al.,* 1993) and the resultant partitionates were evaporated to dryness with a rotary evaporator to yield petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble materials (Table 1). The residues were then stored in a refrigerator until further use.

Evaluation of Biological Activities

Total phenolic content: The total phenolic content of the extractives were 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 through the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

Table 1. Kupchan partitioning of *A. chaplasha* and *B. spectabillis*

Fractions/crude extract	*A. chaplasha* (g)	*B. spectabillis* (g)
Me	5.0	5.0
PESF	1.0	1.5
CTCSF	1.5	1.2
CSF	1.0	0.5
AQSF	0.5	1.0

Thrombolytic activity: The thrombolytic activity was determined by the method developed Prasad *et al*. (2006) where streptokinase (SK) was used as the positive control.

Membrane stabilizing activity: The membrane stabilizing activity of the extractives was assessed through hypotonic solution- and heat-induced hemolysis of human erythrocytes by following standard method (Omale and Okafor, 2008).

Antimicrobial screening: Antimicrobial activity was determined by the commonly used disc diffusion method (Bauer *et al*., 1966).

Brine shrimp lethality bioassay: This technique was applied for determination of the general toxic properties of the DMSO solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay (Meyer *et al*., 1982). Vincristine sulphate was used as positive control.

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

Results and Discussion

The crude extracts of leaves of *A. chaplasha* and *B. spectabillis* as well as their Kupchan partitionates were subjected to assays for total phenolic content, free radical scavenging, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities.

Total phenolic content of the extractives: The total phenolic content of the samples of *A. chaplasha* were found in the range of 7.66 to 85.77 mg of GAE/gm of sample. The highest amount of phenolic compounds (85.77±0.84 mg of GAE/gm of extractive) was observed in the petroleum ether soluble fraction. Among the samples of *B. spectabillis*, the total phenolic content was found in the range of 2.31 to 50.00 mg of GAE/gm of dried extract while the highest amount of TPC (50.00±0.19 mg of GAE/gm of extractive) was found in the petroleum ether soluble fraction (Table 2).

Free radical scavenging activity of the extractives: In the DPPH free radical scavenging assay, the aqueous soluble fraction of crude methanol extract of leaves of *A. chaplasha* revealed maximum free radical scavenging activity (IC_{50} = 15.60±0.27 µg/ml) when compared to ascorbic acid (IC_{50} = 5.8±0.21 µg/ml). Among the test samples of *B. spectabillis*, the crude extract demonstrated the highest free radical scavenging activity (IC_{50} =18.33±0.17 µg/ml) (Table 2).

Table 2. Total phenolic content, cytotoxic, and free radical scavenging activity of *A. chaplasha* and *B. spectabilis*.

Plants	Samples/ Standards	Total phenolic content (mg of GAE/gm of extract)	DPPH Free radical scavenging activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
A. chaplasha	ME	30.15 ± 0.84	15.70 ± 0.21	1.960 ± 0.15
	PESF	85.77 ± 0.84	31.20 ± 0.53	0.781 ± 0.36
	CTCSF	39.78 ± 0.84	62.50 ± 0.81	9.116 ± 0.74
	CSF	7.66 ± 0.26	31.20 ± 0.43	24.56 ± 0.42
	AQSF	61.26 ± 0.23	15.60 ± 0.27	6.416 ± 0.22
B. spectabillis	ME	50.00 ± 0.19	18.33 ± 0.17	3.27 ± 0.28
	PESF	41.81 ± 0.48	19.76 ± 0.84	4.57 ± 0.52
	CTCSF	2.87 ± 0.56	44.74 ± 0.23	4.78 ± 0.37
	CSF	11.37 ± 0.11	49.46 ± 0.45	5.13 ± 0.39
	AQSF	2.31 ± 0.87	22.82 ± 0.88	1.28 ± 0.57
	VS	-	-	0.451
	BHT	-	27.5 ± 0.54	-
	Ascorbic acid	-	5.8 ± 0.21	-

The average values of three calculations are presented as mean ± S.D.; BHT= Butylated hydroxytolune; VS= Vincristine sulfate; ME= Methanolic crude extract; PESF= Petroleum ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction.

Thrombolytic activity: In order to identify the drugs with the ability to promote lysis of blood clot from natural resources, the extractives of *A. chaplasha* and *B. spectabillis* were assessed for thrombolytic activity and the results are presented in Table 3. Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C showed 66.77% lysis of clot. On the other hand, distilled water when treated as negative control, revealed a negligible lysis of clot (3.79%). In this study, different extractives of *A. chaplasha* and *B. spectabillis* demonstrated poor clot lysis ranging from 2.56% to 17.07% and 2.24% to 17.59%, respectively. The chloroform soluble fraction of the methanol extract of *A. chaplasha* and aqueous soluble fraction of the crude extract of *B. spectabillis* showed 17.07±0.23% and 17.59±0.74% clot lysis, respectively (Table 3).

Table 3. Thrombolytic activity of *A. chaplasha* and *B. spectabillis* extractives.

Sample	% Of lysis of RBC	
	A. chaplasha	*B. spectabillis*
ME	2.77±0.54	8.62±0.18
PESF	5.55±0.39	11.50±0.24
CTCSF	13.79±0.48	2.24±0.71
CSF	17.07±0.23	6.01±0.29
AQSF	2.56±0.68	17.59±0.74
Water	3.79 ± 0.55	
Streptokinase	66.77 ± 1.08	

Membrane stabilizing activity of the extractives: The membrane stabilizing activity of *A. chaplasha* and *B. spectabillis* extractives was also determined. All the extractives significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat, as compared to the standard acetyl salicylic acid. In hypotonic solution and heat induced conditions,

the crude methanol extract of *A. chaplasha* inhibited 41.96 ± 0.87% and 40.00 ± 0.78% haemolysis of RBCs, respectively as compared to 72.79% and 42.12% inhibition by acetyl salicylic acid (0.10 mg/ml), respectively (Table 4). The carbon tetrachloride soluble fraction of methanol extract of *B. spectabillis* revealed 65.68 ± 0.36% and 35.62 ± 0.82% inhibition of hypotonic solution and heat induced haemolysis, respectively (Table 4).

Table 4. Percentage inhibition of hypotonic solution- and heat-induced hemolysis of erythrocyte membrane by *A. chaplasha* and *B. spectabillis* extractives

Sample	% Inhibition of haemolysis			
	A. chaplasha		*B. spectabillis*	
	Hyponotic solution induced	Heat induced	Hyponotic solution induced	Heat induced
ME	41.96 ± 0.87	40.00 ± 0.78	9.5 ± 0.08	20.92 ± 0.84
AQSF	32.16 ± 0.09	27.82 ± 0.25	20.22 ± 0.19	22.08 ± 0.21
CSF	34.60 ± 0.27	30.46 ± 0.14	27.94 ± 0.54	29.10 ± 0.64
CTCSF	40.00 ± 0.65	27.58 ± 0.88	65.68 ± 0.36	35.62 ± 0.82
PESF	32.15 ± 0.81	31.70 ± 0.57	61.86 ± 0.68	17.25 ± 0.05
ASA	72.79 ± 0.47	42.12 ± 0.23	72.79 ± 0.47	42.12 ± 0.23

ASA= Acetyl salicylic acid.

Table 5. Antimicrobial activity of *A. chaplasha.*

Test microorganisms	Diameter of zone of inhibition (mm)			
	ME	PESF	CTCSF	Ciprofloxacin
Bacillus cereus	9.0 ± 0.39	-	-	45 ± 2.01
B. megaterium	-	-	-	42 ± 1.17
B. subtilis	7.0 ± 0.27	7.0±0.45	-	42 ± 0.73
Staphylococcus aureus	8.0 ± 0.33	-	8.0 ± 0.38	42 ± 0.23
Sarcina lutea	8.0 ± 0.18	7.0±0.30	10.0 ± 0.04	42 ± 0.56
Escherichia coli	-	-	-	42 ± 0.43
Pseudomonas aeruginosa	-	-	-	42 ± 1.11
Salmonella Typhi	8.0 ± 0.34	-	-	45 ± 0.73
S. Paratyphi	8.0 ± 0.68	-	-	47 ± 2.33
Shigella boydii	8.0 ± 0.82	-	-	34 ± 0.58
Sh. dysenteriae	8.0 ± 0.35	8.0±1.03	13.0 ± 0.43	42 ± 0.22
Vibrio mimicus	8.0 ± 0.67	9.0±0.83	-	40 ± 0.45
V. parahaemolyticus	-	-	8.0 ± 0.69	35 ± 0.44
Saccharomyces cerevisiae	-	-	-	38 ± 0.49
Candida albicans	8.0 ± 0.28	-	-	37 ± 0.33
Aspergillus niger	-	7.0±0.58	-	38 ± 0.11

Antimicrobial activity: The extractives of *A. chaplasha* and *B. spectabillis* were also subjected to screenings for *in vitro* antimicrobial activity against five gram positive and eight gram negative bacteria and three fungi at 400 µg/disc. The test samples of *A. chaplasha* revealed mild to moderate inhibitory activity against the tested pathogens having zone of inhibition within the range of 7.0 to 13.0 mm. The carbon tetrachloride soluble fraction of *A. chaplasha* demonstrated 13±0.43 mm zone of inhibition against *Shigella dysenteriae* (Table 5). Among the samples of *B. spectabillis*, only the chloroform soluble fraction revealed weak antimicrobial activity as evident from smaller zone of inhibition (8.0 to 11.0 mm) against all the tested microorganisms. This fraction also showed 11.0±0.80 and 11.0±0.43 mm zones of inhibition

against *Pseudomonas aeruginosa* and *Bacillus cereus*, respectively (Table 6). The inhibitory activity of the extractives was compared with standard antimicrobial agent, ciprofloxacin.

Table 6. Antimicrobial activity of *B. spectabillis.*

Test microorganisms	Diameter of zone of inhibition (mm)	
	CSF	Ciprofloxacin
Bacillus cereus	11.0 ± 0.43	45.0 ± 2.01
B. megaterium	9.0 ± 0.28	42.0 ± 1.17
B. subtilis	9.0 ± 0.62	42.0 ± 0.73
Sarcina lutea	10.0 ± 0.58	42.0 ± 0.23
Staphylococcus aureus	10.0 ± 0.39	42.0 ± 0.56
Escherichia coli	9.0 ± 0.22	42.0 ± 0.43
Pseudomonas aeruginosa	11.0 ± 0.80	42.0 ± 1.11
Salmonella Typhi	10.0 ± 0.13	45.0 ± 0.73
S. Paratyphi	8.0 ± 0.38	47.0 ± 2.33
Shigella boydii	9.0 ± 0.81	34.0 ± 0.58
S. dysenteriae	9.0 ± 0.31	42.0 ± 0.22
Vibrio mimicus	9.0 ± 0.58	40.0 ± 0.45
V. parahaemolyticus	9.0 ± 0.46	35.0 ± 0.44
Candida albicans	9.0 ± 0.19	38.0 ± 0.49
Aspergillus niger	10.0 ± 0.35	37.0 ± 0.33
Saccharomyces cerevisiae	10.0 ± 0.21	38.0 ± 0.11

CSF = Chloroform soluble fraction

Cytotoxic activity of the extractives: In the brine shrimp lethality bioassay, the petroleum ether soluble fraction of *A. chaplasha* revealed the highest cytotoxic potential having LC_{50} value of 0.781±0.36 µg/ml, whereas the standard vincristine sulphate exhibited an LC_{50} value of 0.451 µg/ml. On the other hand, among the test samples of *B. spectabillis*, the aqueous soluble fraction displayed the highest cytotoxic potential with LC_{50} value 1.28±0.57 µg/ml. This suggested the presence of potent bioactive components in the above mentioned extracts (Table 2).

Conclusion

It is clearly evident from the above findings that the leaves of *A. chaplasha* and *B. spectabillis* have potent antioxidant and cytotoxic properties. The plants also exhibited moderate to strong membrane stabilizing potential. Therefore, these plants are good candidates for further systematic, chemical and biological studies in order to isolate the active principles.

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References

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**, 493-496.

Brand-Williams, W., Cuvelier, M.E., Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol*. **28**, 25-30.

Butler, M.S. 2005. Natural products to drugs: natural product derived compounds in clinical trials. *Nat. prod. Rep*. **22**, 162-195.

Flora of China, 2012. http://www.floracafe.com/Search_PhotoDetails.aspx?Photo=Top&Id=270

Gan, R., Kuang, L., Xu, X., Zhang, Y., Xia, E., Song, F. and Li, H. 2010. Screening of natural antioxidants from chinese medicinal plants associated with treatment of rheumatic diseases. *Molecules* **15**, 5988-5997

Harbertson, J. and Spayd, S. 2006. Measuring phenolics in the winery. *Am. J. Enol. Vitic*. **57**, 280-288.

Jawla, S. 2011. Hypoglycemic potential of *Bougainvillea spectabillis* root bark in normal and alloxan induced diabetic rats. *Pharmacol. online*. **3**, 73-87.

Kaisar, M.A., Rahman, M.S., Rahman, M.Z., Hasan, C.M. and Rashid, M.A. 2011. A review on phytochemicals from some medicinal plants of Bangladesh. *J. Phar. Nutri. Sci*. **1**, 87-95.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, J.B., Nicholsand, D.E. and Mclaughlin, J.L. 1982. Brine shrimp; a convenient general bioassay for active plant constituents. *Planta Med*. **45**, 31-34.

Omale, J. and Okafor, P.N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenols composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr. J. Biotechnol.* **7**, 3129-3133.

Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G.M. and Daginawala, H.F. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thrombosis J.* **4**, 14.

.Saikia, H. and Lama A. 2011. Effect of *Bougainvillea spectabilis* leaves on serum lipids in albino rats fed with high fat diet. *Int. J. Pharm. Sci. Drug Res*. **3**, 141-145.

Sikder, M.A., Rahman, M.A., Kaisar, M.A., Rahman, M.S., Hasan, C.M. and Rashid, M.A. 2011. *In vitro* antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of seeds of *Syzygium cumini* L. *Lat. Am. J. Pharm.* **30**, 781-785.

Van Wagenen, B.C., Larsen, R., Cardellina, J.H. II, Ran dazzo, D., Lidert, Z.C., and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem*. **58**, 335-337.