

Bioactivities of *Malvaviscus arboreus* var. *drummondii* and *Phyllanthus reticulatus* Poir.

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ABSTRACT: The study was designed to evaluate the bioactivities of crude methanol extracts of leaves of *Malvaviscus arboreus* and *Phyllanthus reticulatus* along with their pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates. The antioxidant potential was evaluated by DPPH and Folin-Ciocalteu reagents using butylated hydroxytoluene (BHT) and ascorbic acid as standards. In the DPPH free radical scavenging assay, the crude methanol extract of *M. arboreus* revealed the highest free radical scavenging activity with IC₅₀ values 6.47 ± 0.78 µg/ml. In brine shrimp lethality bioassay, the chloroform soluble materials of *M. arboreus* displayed the highest cytotoxic potential having LC₅₀ values 2.73 ± 0.36 µg/ml, whereas the standard vincristine sulphate exhibited an LC₅₀ value of 0.45 µg/ml. In the thrombolytic activity assay, the pet-ether soluble fractions of *M. arboreus* and *P. reticulatus* revealed 55.60 ± 0.48 % and 18.99 ± 0.84% clot lysis, respectively. In the membrane stabilizing activity assay, the chloroform soluble fraction of *M. arboreus* inhibited heat- and hypotonic solution- induced haemolysis of RBCs by 54.60 ± 0.81 % and 66.96 ± 0.84 % as compared to 57.06 % and 72.79 % inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. On the other hand, the pet-ether soluble materials of crude methanol extract of *P. reticulatus* demonstrated 57.11 ± 0.57 % and 70.62 ± 0.22 % inhibition of heat- and hypotonic solution- induced hemolysis, respectively.

Key words: *Malvaviscus arboreus*, *Phyllanthus reticulatus*, antioxidant, DPPH, cytotoxicity, thrombolysis, membrane stabilization

INTRODUCTION

According to the World Health Organization (WHO), 80% of the world's populations rely on traditional medicines.¹ The practice of herbal medicines is common in rural areas where western medicines are either too expensive or not available.¹ Plants have been used frequently to treat common infectious diseases and some of these traditional medicines are still in use. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pubmed during the period between 1966 and 1994, but in the following decade, between 1995 and 2004, 307 were published.² The demand for more and more drugs

from plant sources is increasing and therefore it is essential for systematic evaluation of plants used in traditional medicines. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources correspond to 78% of the new drugs approved by the FDA between 1983 and 1994.³ Hence, there is need to screen medicinal plants with promising biological activity.⁴

Malvaviscus arboreus var. *drummondii* (Synonyms: *Malvaviscus balbisii* DC., *Hibiscus malvaviscus* L. Bengali name: Lanka jaba) belongs to the hibiscus family, Malvaceae. It is a species of the flowering plants that is native to the Southeastern United States, Mexico and Central and South America. Liniment made from the base of

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the plant is used for dressing burns. The leaf paste is applied and rubbed in the burnt area.⁵

Phyllanthus reticulatus Poir. (Synonyms: *Anisonema reticulatum* Poir. A. Juss., *Cicca reticulata* Poir. Kurz.; Bengali name: Panjuli, Panseuli, Chitki, Pankushi) belonging to the family Euphorbiaceae, is a large often scandent shrub. It grows throughout the country in fallow lands. The fruits have been reported to exhibit anti-inflammatory activity.⁶ Crude methanol extract of the whole plant possesses free radical scavenging activity.⁷ Its leaf and stem have been reported to contain lupeol.⁸ A new purine derivative, 3-(3-methylbut-2-en-1-yl) isoguanine and a new cleistanthane-type diterpenoid glucoside, 19-hydroxyspruceanol 19-*O*- β -D-glucopyranoside, together with eight known compounds have been isolated from *P. reticulatus*.⁹

As part of our ongoing investigations on medicinal plants of Bangladesh¹⁰⁻¹², the extractives of leaves of *M. arboreus* were screened for the antioxidant potential in terms of total phenolic content and free radical scavenging activities; cytotoxic, thrombolytic and membrane stabilizing activities whereas the extractives of *P. reticulatus* were studied for thrombolytic and membrane stabilizing activities for the first time and we, here in, report the results of our preliminary investigations.

MATERIALS AND METHODS

Collection of plant materials and extraction.

The leaves of *M. arboreus* and *P. reticulatus* were collected in March 2012 from National Botanical Garden, Mirpur, Dhaka and voucher specimens (DUSH-10780 and DUSH-10781, respectively) for these collections have been maintained in Dhaka University Salar Khan Herbarium for future references.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 gm each) of both the plants were separately soaked in 2.0 liters of methanol at room temperature for 7 days. The extracts were filtered through fresh cotton bed and finally with Whatman filter paper number 1. The

filtrates were concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 gm) of each of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol¹³ and the resultant partitionates were evaporated to dryness with a rotary evaporator to yield pet-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.

Table 1. Kupchan partitionates of *M. arboreus* and *P. reticulatus*.

Crude extract/ Fraction	<i>M. arboreus</i> (gm)	<i>P. reticulatus</i> (gm)
ME	5.0	5.0
PESF	0.5	1.0
CTCSF	1.5	1.5
CSF	1.0	0.5
AQSF	1.5	1.3

ME= Methanolic crude extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction.

DPPH free radical scavenging assay. Following the method developed by Brand-Williams *et al.*¹⁵, the antioxidant activity of the test samples was assessed by evaluating the scavenging of stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

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).¹⁴

Brine shrimp lethality bioassay. This was applied for the determination of general toxic properties of dimethylsulfoxide (DMSO) solution of plant extractives against *Artemia salina* in a one day assay.¹⁶ Vincristine sulphate was used as positive control.

Thrombolytic activity. The method developed by Prasad *et al.*¹⁷ was used to determine the thrombolytic activity where streptokinase (SK) was used as positive control.

Membrane stabilizing activity. Inhibition of heat and hypotonic solution-induced haemolysis of human erythrocytes by the extractives was used to

Table 2. Total phenolic content, free radical scavenging and cytotoxic activities of *M. arboreus*.

Plants	Samples/ Standards	Total phenolic content (mg of GAE/gm of extract)	DPPH Free radical scavenging activity (IC ₅₀ µg/ml)	Cytotoxicity (LC ₅₀ µg/ml)
<i>M. arboreus</i>	ME	20.06 ± 0.87	6.47 ± 0.78	3.82 ± 0.08
	PESF	0.13 ± 0.09	42.19 ± 0.25	28.46 ± 0.19
	CTCSF	18.13 ± 0.27	11.44 ± 0.14	53.97 ± 0.54
	CSF	18.38 ± 0.65	10.73 ± 0.88	2.73 ± 0.36
	AQSF	12.50 ± 0.81	11.59 ± 0.57	4.86 ± 0.68
	VS	-	-	0.45
	BHT	-	27.50 ± 0.54	-
	Ascorbic acid	-	5.80 ± 0.21	-

ME= Methanolic crude extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction; BHT= Butylated hydroxytoluene; VS= Vincristine sulfate.

Table 3. Thrombolytic activity of *M. arboreus* and *P. reticulatus* extractives.

Sample	Clot lysis	
	<i>M. arboreus</i>	<i>P. reticulatus</i>
ME	12.99 ± 0.19	4.82 ± 0.17
PESF	55.60 ± 0.48	18.99 ± 0.84
CTCSF	18.74 ± 0.56	15.98 ± 0.23
CSF	42.33 ± 0.11	9.30 ± 0.45
AQSF	20.69 ± 0.87	17.89 ± 0.88
Water		3.79 ± 0.55
SK		66.77 ± 1.08

SK = Streptokinase (Positive control); Water (negative control)

Table 4. Percentage inhibition of hypotonic solution and heat- induced hemolysis of RBC by *M. arboreus* and *P. reticulatus* extractives.

Sample	% Inhibition of haemolysis			
	<i>M. arboreus</i>		<i>P. reticulatus</i>	
	Hypnotic solution-induced	Heat-induced	Hypnotic solution-induced	Heat-induced
ME	54.26 ± 0.84	47.62 ± 0.21	42.09 ± 0.15	47.65 ± 0.28
PESF	64.97 ± 0.23	49.00 ± 0.27	70.62 ± 0.22	57.11 ± 0.57
CTCSF	50.28 ± 0.26	39.56 ± 0.43	41.56 ± 0.42	40.86 ± 0.39
CSF	66.96 ± 0.84	54.60 ± 0.81	43.65 ± 0.74	50.51 ± 0.37
AQSF	53.20 ± 0.84	45.31 ± 0.53	47.77 ± 0.36	51.28 ± 0.52
Acetyl salicylic acid	72.79 ± 0.47	57.06 ± 0.23	72.79 ± 0.47	57.06 ± 0.23

assess the membrane stabilizing activity by a previously developed method.¹⁸

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 extractives of leaves of *M. arboreus* were studied for antioxidant, cytotoxic, thrombolytic and membrane stabilizing activities whereas that of *P. reticulatus* were subjected to assays for thrombolytic and membrane stabilizing properties.

The total phenolic content of the samples of *M. arboreus* was found in the range of 0.13 to 20.06 mg of GAE/g of extractives, with the highest amount of phenolic compounds (20.06 ± 0.87 mg) being observed in the crude methanol extract (Table 2). As the phenolics are relatively polar in nature, thus their presence in the methanol extract is justified.

In the DPPH free radical scavenging assay, the crude methanol extract of leaves of *M. arboreus* revealed maximum free radical scavenging activity (IC₅₀ = 6.47 ± 0.78 µg/ml) when compared to ascorbic acid (IC₅₀=5.80 ± 0.21 µg/ml) (Table 2).

In the brine shrimp lethality bioassay, the chloroform soluble materials of *M. arboreus* displayed the highest cytotoxic potential having LC₅₀ value 2.73 ± 0.36 µg/ml as compared to 0.45 µg/ml for the standard vincristine sulphate. This suggested the presence of potent bioactive components in the above mentioned test sample (Table 2).

The extractives of *M. arboreus* and *P. reticulatus* were assayed for thrombolytic activity to determine the ability of natural products to promote lysis of blood clot. Addition of 100 µl streptokinase (SK), a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37° C produced 66.77% lysis of the clot. On the other hand, distilled water treated as negative control, revealed a negligible lysis of clot (3.79%). In this study, different extractives of *M. arboreus* and *P. reticulatus* demonstrated clot lysis activity ranging from 12.99 % to 55.60 % and 4.82 % to 18.99 %, respectively. The pet-ether soluble fractions of crude methanolic extracts of *M. arboreus* and *P. reticulatus* revealed 55.60 ± 0.48 % and 18.99 ± 0.84 % clot lysis, respectively (Table 3).

The membrane stabilizing activity of *M. arboreus* and *P. reticulatus* extractives was also determined. Both significantly protected the lysis of human erythrocyte membrane induced by heat and hypotonic solution, as compared to the standard acetyl salicylic acid. In heat- and hypotonic solution-induced conditions, the chloroform soluble partitionates of *M. arboreus* inhibited 54.60 ± 0.81 % and 66.96 ± 0.84 % haemolysis of RBCs, respectively as compared to 57.06 % and 72.79% inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. The pet-ether soluble fraction of crude methanol extract of *P. reticulatus* demonstrated 57.51 ± 0.57 % and 70.62 ± 0.22 % inhibition of hypotonic solution- and heat-induced hemolysis, respectively (Table 4).

It is clearly evident from the above findings that the leaves of *M. arboreus* have significant free radical scavenging, cytotoxic and thrombolytic properties. On the other hand, the leaves of *P. reticulatus* have significant membrane stabilizing

activity. The plant also exhibited mild thrombolytic potential. Traditional healers of southern districts of Noakhali and Feni of Bangladesh use leaves and flowers of *M. arboreus* for skin diseases of hands¹⁹ while the stem of *P. reticulatus* is used in the treatment of diarrhea²⁰, chicken pox²¹ and dental carries.²² The findings of this study especially the low LC₅₀ values in brine shrimp lethality bioassay justify the traditional uses of these two plant species. Therefore, both plants are potential candidates for further chemical investigations to isolate the bioactive molecules.

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