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EVALUATION OF THROMBOLYTIC AND MEMBRANE STABILIZING ACTIVITIES OF FOUR MEDICINAL PLANTS OF BANGLADESH

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Abrus precatorius L., *Magnolia pterocarpa* Roxb., *Dracaena spicata* Roxb., *Ravenala madagascariensis* Sonn., thrombolysis, membrane stabilization

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ABSTRACT: The crude methanol extracts of aerial parts of *Abrus precatorius* L., leaf *of Magnolia pterocarpa* Roxb. and *Dracaena spicata* Roxb. and leaf and bark of *Ravenala madagascariensis* Sonn. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing activities. Among the extractives of *A. precatorius*, the crude methanol extract exhibited the highest thrombolytic activity (34.92±0.54 %) while the carbon tetrachloride soluble fraction of *M. pterocarpa* exhibited 22.59±0.88 % clot lysis. *D. spicata* extractives showed mild thrombolytic activity. The methanolic crude extract of *R. madagascariensis* leaf and the aqueous soluble fraction of *R. madagascariensis* bark extract showed 45.32±0.82 % and 32.67±0.74% clot lysis, respectively. In hypotonic solution and heat induced conditions, the crude methanol extract of *A. precatorius* and the hexane soluble fraction of crude methanol extract of *M. pterocarpa* inhibited 63.46±0.84 % & 36.54±0.21 % and 66.12±0.66 % & 40.54±0.02 % haemolysis of RBCs, respectively as compared to 71.90 % and 42.12 % inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. The crude methanol extract of *D. spicata* demonstrated 64.44±0.68 % and 36.52±0.19 % inhibition of hypotonic solution and heat induced hemolysis, respectively. The chloroform soluble fraction of *R. madagascariensis* leaf extract demonstrated 28.72±0.61 % & 39.97±0.39 % and the hexane soluble fraction of *R. madagascariensis* bark extract revealed 53.78±0.17 % & 41.83±0.61 % inhibition of hypotonic solution and heat induced hemolysis of RBCs, respectively.

INTRODUCTION: According to the World Health Organization (WHO), 80% of the world's populations rely on traditional medicines.[1] The practice of herbal medicine is common in rural areas where western medicines are too expensive or not available.[1]

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Humans have frequently used plants to treat common infectious diseases and some of these traditional medicines are still part of the habitual treatment of various maladies.

It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pubmed during the period between 1966–1994, but in the following decade, between 1995 and 2004, 307 were published [2]. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments.

Hence, there is need to screen medicinal plants for promising biological activity [3]. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994 [4].

Abrus precatorius L. (Synonyms: *Abrus abrus, Glycine abrus*; Bengali name: Kunch, Ratii) commonly known as crab's eye, john crow bead, precatory bean and jumbie bea, is a slender, perennial climber of Fabaceae family. The plant is native to India and other tropical and subtropical areas of the world. A tea is made from the leaves and used to treat fevers, cough and cold. Seeds are poisonous and therefore are used after mitigation. The plant is also used in Ayurveda.[5] An ethanolic extract of seeds of *A. precatorius* was found to have antioxidant, anti-inflammatory and analgesic potentials in rodents [6].

A. precatorius seed extract also caused reversible alterations in the estrous cycle pattern and completely blocked ovulation in Sprague-Dawley rats [7]. A methanolic extract of the plant produced dose-dependent bronchodilator activity in a guinea pig model [8].

Magnolia pterocarpa Roxb. (Synonyms: *Lirianthe grandiflora, Liriodendron grandiflorum* Roxb., *L. indicum* Spreng.) locally known as dulichapa, is a flowering medium to large tree of Magnoliaceae family. The plant is native to 3600 feet altitude forests of India, Burma and Pakistan. The bark contains sesamin, eudesmin, fargesin, imperatorin, dimethyl teraphthalate and β-sitosterol. Powdered bark is used for fever and cough [9].

Dracaena spicata Roxb. (Synonyms: *D. wallichii* Kunth., *Draco spicata* Roxb. Kuntze; Bengali name: ognikundo), commonly known as dragon tree, is a tree of Asparagaceae family. The plant is distributed in Assam, Bangladesh, Andaman Islands and Myanmar. The leaf extract is used by the chakma communities in the treatment of measles [10].

Leaf juice is used to cure long term fever, coughs and mucus in nose by traditional healers of the Marma tribe of Naikhongchhari, Bandarban District [11].

Ravenalamadagascariensis Sonn. (Synonyms: - *Heliconia ravenala* Willemet, *Urania madagas-cariensis* Sonn. Raeusch; Bengali name: Panthapadak) commonly known as Traveler's Tree or Traveler's Palm, is a species from Madagascar. It is not a true palm but a member of the bird of paradise family, Strelitziaceae. It is endemic to secondary forests in Madagascar. The leaves have been reported to have anti-diabetic activity in alloxan induced diabetic rats [12].

As part of our ongoing investigations on medicinal plants of Bangladesh [13, 14, 15, 16, 17, 18], the crude methanol extracts of aerial parts of *A. precatorius*, leaf *of M. pterocarpa* and *D. spicata* and leaf and bark of *R. madagascariensis* growing in Bangladesh, as well as their organic and aqueous soluble fractions 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 aerial parts of *A. precatorius,* leaf *of M. pterocarpa* and *D. spicata* and leaf and bark of *R. madagascariensis* were collected in March 2012 from Dhaka. Voucher specimens DUSH-10775, DUSH-10774 and DUSH-10777 for collection of *A. precatorius, M. pterocarpa* and *D. spicata* have been deposited in Salar Khan Herbarium, Department of Botany, University of Dhaka, respectively. In Bangladesh National Herbarium, voucher specimen DACB 38302 has been deposited for the collection of leaf and bark of *R. madagascariensis.* The experiments were conducted in Phytochemical Research Laboratory, State University of Bangladesh in 2012.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 g each) of the collected plants were separately soaked in 2.0 liters of methanol at room temperature for seven days. The extracts were then 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 partition protocol [19] and the resultant partitionates were evaporated to dryness

with rotary evaporator to yield hexane (HXSF), 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 PARTITIONING OF *A. PRECATORIUS, M. PTEROCARPA, D. SPICATA* AND *R. MADAGASCARIENSIS*

Crude extract/ Fractions	*A. precatorius* (g)	*M. pterocarpa* (g)	*D. spicata* (g)	*R. madagascariensis* Leaf (g)	*R. madagascariensis* Bark (g)
ME	5.0	5.0	5.0	5.0	5.0
HXSF	1.0	1.3	1.0	1.5	1.0
CTCSF	1.5	0.8	1.0	1.2	0.5
CSF	1.0	0.5	0.5	0.5	1.5
AQSF	0.5	1.5	1.5	1.0	0.8

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

Thrombolytic activity: The thrombolytic activity was evaluated by the method developed by Prasad *et al* (2006) [20] by using streptokinase 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) [21].

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 methanol extracts of aerial parts of *A. precatorius*, leaf *of M. pterocarpa* and *D. spicata* and leaf and bark of *R. madagascariensis* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing potentials. In order to identify the drugs with the ability to promote lysis of blood clot from natural resources, the extractives of *A. precatorius*, *M. pterocarpa*, *D. spicata* and *R. madagascariensis* were assessed for thrombolytic activity. Addition of 100 µl streptokinase, 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, treated as negative control, revealed a negligible lysis of clot (3.79 %).

Among the extractives of *A. precatorius* and *M. pterocarpa*, the crude methanol extract and the carbon tetrachloride soluble fraction exhibited 34.92±0.54 % and 22.59±0.88 % clot lysis, respectively. On the other hand, *D. spicata* extractives showed mild thrombolytic activity and the highest thrombolytic activity was demonstrated by the carbon tetrachloride soluble fraction (21.05±0.23 %). Different extractives of *R. madagascariensis* leaf and bark demonstrated mild to moderated clot lysis activity ranging from 22.55 % to 45.32 % and 24.44 % to 32.67 %, respectively. The methanolic crude extract of leaf and the aqueous soluble fraction of the bark extract showed 45.32±0.82 % and 32.67±0.74 % clot lysis, respectively (**Table 2**).

TABLE 2: THROMBOLYTIC ACTIVITIES OF *A. PRECATORIUS, M. PTEROCARPA, D. SPICATA* AND *R. MADAGASCARIENSIS* LEAF AND BARK EXTRACTIVES

Samples/ Standard	*A. precatorius*	*M. pterocarpa*	*D. spicata*	*R. madagascariensis*	
				Leaf	Bark
ME	34.92±0.54	9.22±0.52	20.23±0.17	45.32±0.82	31.21±0.18
HXSF	18.63±0.39	2.28±0.63	6.74±0.84	22.55±1.41	24.44±0.24
CTCSF	17.72±0.48	22.59±0.88	21.05±0.23	44.28±0.39	25.58±0.71
CSF	4.83±0.23	2.56±1.03	0.39±0.45	29.44±0.21	27.13±0.29
AQSF	4.73±0.68	15.72±0.41	17.56±0.88	29.77±0.07	32.67±0.74
Water			3.79±0.55		
SK			66.77±1.08		

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

The membrane stabilizing activity of *A. precatorius*, *M. pterocarpa*, *D. spicata* and *R. madagascariensis* extractives was also determined. In hypotonic solution and heat induced conditions, the crude methanol extract of *A. precatorius* inhibited 63.46±0.84 % and 36.54±0.21 % haemolysis of RBCs, respectively as compared to 71.90 % and 42.12 % inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. The hexane soluble fraction of crude methanol extract of *M. pterocarpa* and the crude methanol extract of *D. spicata* demonstrated 66.12±0.66 % & 40.54±0.02 % and 64.44±0.68 % & 36.52±0.19 % inhibition of hypotonic solution and heat induced hemolysis, respectively.

The chloroform soluble fraction of *R. madagascariensis* leaf extract demonstrated 28.72±0.61 % & 39.97±0.39 % and the hexane soluble fraction of *R. madagascariensis* bark extract revealed 53.78±0.17 % & 41.83±0.61 % inhibition of hypotonic solution and heat induced hemolysis of RBCs, respectively (**Table 3**).

TABLE 3: MEMBRANE STABILIZING ACTIVITIES OF *A. PRECATORIUS, M. PTEROCARPA, D. SPICATA* AND *R. MADAGASCARIENSIS* LEAF AND BARK EXTRACTIVES

Plants	Samples/ Standards	% Inhibition of haemolysis	
		Hypnotic solution induced	Heat induced
A. precatorius	**ME**	63.46±0.84	36.54±0.21
	HXSF	7.18±0.84	31.87±0.53
	CTCSF	8.86±0.84	23.24±0.81
	CSF	17.40±0.26	34.47±0.43
	AQSF	26.90±0.23	19.62±0.27
M. pterocarpa	**ME**	47.75±0.85	37.12±1.07
	HXSF	66.12±0.66	40.54±0.02
	CTCSF	57.30±0.15	35.75±0.57
	CSF	44.12±0.63	36.86 ±0.32
	AQSF	52.39±0.26	39.39±0.47
D. spicata	**ME**	64.44±0.68	36.52±0.19
	HXSF	34.71±0.33	34.97±0.48
	CTCSF	60.92±0.47	26.22±0.56
	CSF	38.04±0.83	12.09±0.11
	AQSF	26.20±0.61	14.86±0.87
R. madagascariensis **Leaf**	**ME**	18.44±0.91	32.57±0.22
	HXSF	9.90±0.87	29.97±0.57
	CTCSF	17.10±0.77	37.15±0.34
	CSF	28.72±0.61	39.97±0.39
	AQSF	18.63±0.26	28.01±0.47
R. madagascariensis **Bark**	**ME**	36.21±0.91	21.31±0.04
	HXSF	53.78±0.17	41.83±0.61
	CTCSF	30.14±0.01	40.94±0.53
	CSF	18.53±0.32	23.24±0.49
	AQSF	28.22±0.46	24.24±0.21
Acetyl salicylic acid		71.90±0.78	42.12±0.38

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

CONCLUSION: The objective of the study was to evaluate the thrombolytic and membrane stabilizing potentials of crude methanol extracts of aerial parts of *A. precatorius*, leaf *of M. pterocarpa* and *D. spicata* and leaf and bark of *R. madagascariensis* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates. It is clearly evident from the above findings that the extractives of *A. precatorius, M. pterocarpa*, *D. spicata* and *R. madagascariensis* bark exhibited mild to moderate thrombolytic activity but the *R. madagascariensis* leaf extractives demonstratd significant thrombolytic activity.

On the other hand, the extractives of *A. precatorius*, *M. pterocarpa*, *D. spicata* and *R. madagascariensis* bark exhibited significant membrane stabilizing activity but the *R. madagascariensis* leaf extractives demonstrated mild to moderate membrane stabilizing activity. Therefore, these plants are good candidates for further systematic, chemical and biological studies to isolate the active principles.

REFERENCES:

1. Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU and Wufem BM: An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. Journal of Ethnopharmacology 2004; 99: 1-4.
2. Rios JL and Recio MC: Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 2005; 100: 80 – 84.
3. Chowdhury JA, Islam MS, Asifuzzaman Sk and Islam MK: Antibacterial and cytotoxic activity screening of leaf extracts of *Vitex negundo* (Fam: Verbenaceae). Journal of Pharmaceutical Science & Research 2009; 1(4): 103-108.
4. Cragg GM, Newman DJ and Snader KM: Natural products in drug discovery and development. Journal of Natural Product 1997; 60: 52-60.
5. Verma D, Tiwari SS, Srivastava S and Rawat A: Pharmacognostical evaluation and phytochemical standardization of *Abrus precatorius* L. seeds. Natural Product Sciences 2011; 17(1): 51–57.
6. Arora R, Gill NS, Kaur S and Jain AD: Phytopharmacological evaluation of ethanolic extract of the seeds of *Abrus precatorius linn*. Journal of Pharmacology and Toxicology 2011; 6 (6): 580–588.
7. Okoko II, Osinubi AA, Olabiyi OO, Kusemiju TO, Noronha CC and Okanlawon AO: Antiovulatory and anti-implantation potential of the methanolic extract of seeds of *Abrus precatorius* in the rat. Endocrine Practice 2010; 16 (4): 554–560.
8. Mensah AY, Bonsu AS and Fleischer TC: Investigation of the bronchodilator activity of *Abrus precatorius*. International Journal of Pharmaceutical Sciences Review & Research 2011; 6 (2): 9–13.
9. Indian medicinal plants/ http: //indian-medicinal-plants.blogspot.com/2009/06/m-maba-nigrescens-dalz.html (accessed on: 21/05/2013).
10. Khisha T, Karim R, Chowdhury SR and Banoo R: Ethnomedical Studies of Chakma Communities of Chittagong Hill Tracts, Bangladesh. Bangladesh Pharmaceutical Journal 2012; 15(1): 59-67.
11. Rahmatullah M, Hossan MS, Hanif A, Roy P, Jahan R, Khan M, Chowdhury MH and Rahman T: Ethnomedicinal Applications of Plants by the Traditional Healers of the Marma Tribe of Naikhongchhari, Bandarban District, Bangladesh. Advances in Natural & Applied Sciences 2009; 3(3): 392-406.
12. Sakthi PS, Vadivu R and Jayshree N: In vitro and In vivo antidiabetic activity of the leaves of *Ravenala madagascariensis* Sonn., on alloxan induced diabetic rats. Journal of Pharmaceutical Science and Technology 2012; 2(9): 312.
13. Sharmin T, Islam F, Kaisar MA, Uddin MG and Rashid MA: Antioxidant, Thrombolytic and Cytotoxic Activities of *Picrasma javanica.* Dhaka University Journal of Pharmaceutical Sciences 2012; 11: 71-74.
14. Hossain SM, Islam F, Sharmin T, Sheikh H, Hasan AMR and Rashid MA: In vitro Antioxidant, Membrane Stabilizing and Thrombolytic Activities of *Glycosmis arborea*. Bangladesh Pharmaceutical Journal 2012; 15(2): 141-143.
15. Sarker R, Sharmin T, Chowdhury SR and Islam F: Thrombolytic Activity and Preliminary Cytotoxicity of Five Different Fractions of Methanol Extract of *Allamanda cathartica* Leaf. Journal of Applied Pharmaceutical Science 2012; 2(7): 129-132.
16. Islam F, Chowdhury SR, Sharmin T, Uddin MG, Kaisar MA and Rashid MA: In Vitro Membrane Stabilizing and Thrombolytic Activities of *Ophirrhiza mungos*, *Mussaenda macrophylla*, *Gmelina philippensis* and *Synedrella nodiflora* Growing in Bangladesh Journal of Pharmacy and Nutrition Science 2013; 3: 71-75.
17. Mita TA, Shihan MH, Rahman M, Sharmin T, Maleque M, Alvi MRH and Chowdhury SR: In Vitro Antioxidant, Cytotoxic, Thrombolytic, Antimicrobial and Membrane Stabilizing Activities of *Murraya paniculata.* American Journal of Research Communication 2013; 1(5): 226-237.
18. Sharmin T, Islam F, Sikder MA, Kabir S, Haque MR and Rashid MA: Membrane Stabilizing and Preliminary Hypoglycemic Activities of *Picrasma javanica*. Bangladesh Pharmaceutical Journal 2013; 16(1): 89-92.
19. Vanwagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC and Swithenbank C: Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. Journal of Organic Chemistry 1993; 58: 335-337.
20. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM and Daginawala HF: Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis, BMC Complementary and Alternative Medicine 2007; 7:36 doi: 10.1186/1472-6882-7-36.
21. Omale J and Okafor PN: Comparative antioxidant capacity, membrane stabilization, polyphenols composition and cytotoxicity of the leaf and stem of *Cissus multistriata.* African Journal of Biotechnology 2008; 7: 3129-3133.

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