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Studies of Biological Activities of the Roots of *Bombax ceiba* L.

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Abstract

Bioactivities of the methanolic crude extract of root of *Bombax ceiba* L. and its organic and aqueous soluble partitionates were studied to investigate its medicinal importance. In Free radical scavenging assay by DPPH method, the aqueous soluble partitionate (AQSF) demonstrated the highest free radical scavenging activity with IC_{50} value of 3.33 ± 0.25 µg/ml. The brine shrimp lethality bioassay of different partitionates of the root demonstrated the significant lethality by the hexane soluble partitionate (HSF) having LC_{50} value of 1.19 ± 0.10 µg/ml. Among different fractionates of the root, the highest percentage (44.55 ± 0.12%) of clot lysis was exhibited by AQSF. The crude extract revealed promising antidiarrheal, hypoglycemic, central and peripheral analgesic activities at both doses of 200- and 400-mg/kg body weight in Swiss-Albino rat model.

Key words: *Bombax ceiba*, antioxidant, thrombolysis, diarrhea, hypoglycemic, analgesic.

Introduction

Bombax is a genus, comprising some 60 species of topical trees in the Bombacaceae family. They are native to western Africa, the Indian subcontinent, Southeast Asia, as well as subtropical regions of East Asia and northern Australia. *Bombax ceiba* L. (syn. *Bombax malabaricum* DC. *Salmalia malabarica*), a medium sized deciduous tree, commonly known as Silk Cotton Tree, Indian Red Kapok tree, Semal, Shimul, Shalmali etc. is also distributed in temperate and tropical Asia (Wang *et al.*, 2013; Verma *et al.*, 2011). It is used in folk medicine for its properties such as demulcent, diuretic, restorative, aphrodisiac and emetic (Faizi and Ali, 1999). It also demonstrated strong anti-*Helicobacter pylori* activity (Wang and Huang, 2005). Its different parts including flowers, roots, stem barks and leaves, etc. have been used for the treatment of diarrhea, dysentery, hepatitis, lymph adenoma and menorrhagia in Taiwan and Kwangton (Ali *et al.*, 2011). Recent study has revealed that the plant possesses strong anti-inflammatory, immunomodulatory, antineoplastic, antioxidant, anti-cancer, hypotensive, hypolipidemic and anti-hyperglycemic activities (Wang *et al.*, 2013)

Considering its medicinal importance to the traditional healers the plant was chosen for the investigation of bioactivities. The roots of *B. ceiba* were subjected to different bioassays to determine its biological activities with the aim of establishing the pharmacological basis for its folkloric use against different diseases.

Materials and Methods

Chemicals and reagents: Gallic acid, tert-butyl-1-hydroxytoluene (BHT), streptokinase (Beacon pharmaceutical Ltd.), acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), normal saline (Beximco Infusion Ltd.), diclofenac sodium, glibenclamide and loperamide (Square Pharmaceuticals) were used in this investigation.

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Collection and extraction of plant material: The roots of *B. ceiba* were collected from Natore, Bangladesh in April, 2016 and taxonomic identification was made at the Department of Botany, University of Dhaka. The roots were first dried at room temperature and then in an oven below 40°C. The dried roots were ground to a coarse powder with a grinder (Cyclotec 200 meshes) and the powder was stored in an airtight bottle used throughout the investigation.The powdered root (1000 g) was soaked in 3.0 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated at 40°C with a rotary evaporator. A portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al*., 1993) into hexane (HSF), dichloromethane (DCMSF), ethylacetate (EASF) and aqueous (AQSF) soluble materials.

Experimental animal: Swiss-Albino mice of either sex aged 4-5 weeks were used for the experiment. The average weight of the mice was 20-25 g. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments. The mice were kept in standard environmental condition (at 24.0 ± 2°C temperature and 60–70% relative humidity and 12 hrs light/12 hrs dark cycle) for a week for acclimatization after their purchase and fed with rodent feed collected from the International Centre for Diarrheal Diseases and Research, Bangladesh (Icddr'b) and water *ad libitum.* The experimental animals were randomly divided into four groups (I to IV) consisting of five mice in each group for each bioassay: positive control, negative control and two test groups receiving methanol extract (ME) at doses of 200-(ME1) and 400- mg/kg of body weight (ME2).

Total phenolic control analysis: Total phenolic content of *B. ceiba* was determined using Folin-Ciocalteu reagent as oxidizing agent following the method described by M. Miah *et al*. (2018).

Determination of free radical scavenging activity: The free radical scavenging activities of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.* (1995). The antioxidant potential was assessed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT).

Brine shrimp lethality bioassay: The brine shrimp lethality activity of extracts of the roots of *Bombax ceiba* L. was determined by following the method illustrated by Meyer *et al*. (1982). Vincristine sulfate was used as reference.

Thrombolytic activity: The thrombolytic activity of all extractives was carried out as per the method reported by Prasad *et al.* (2007).

Antidiarrheal activity: The antidiarrheal activity of the methanol extract of *B. ceiba* roots was evaluated through castor oil induced diarrhea in mice (Shoba & Thomas, 2001), where loperamide was used as reference drug.

Hypoglycemic activity: The lowering of blood glucose level of the experimental animals was measured by tail tipping method (Hasan *et al.*, 2015) on Swiss-Albino mice using glibenclamide as standard drug.

Central analgesic activity: The central analgesic activity was determined by tail immersion method following the method described by Chakraborty *et al.* (2018) using diclofenac sodium as a positive control.

Peripheral analgesic activity: Peripheral analgesic activity was evaluated by determining the ability of the test samples to inhibit acetic-acid induced abdominal writhing in mice (Razan *et al.*, 2016).

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

Results and Discussion

The amount of total phenolic content in different extractives of roots of *B. ceiba* ranged from 85.32 ± 0.26 mg to 168.99 ± 1.02 mg of GAE (Gallic acid equivalent)/g of extractives. Among all the

extractives, the highest phenolic content was found in AQSF (168.99 ± 1.02 mg GAE/ g of extractives).

Table 1. Total phenolic content of the crude extract and its different partitionates of *B. ceiba*.

Plant part	Sample code	Total phenolic content (mg of GAE/g of extractives)
Root	ME	85.32 ± 0.26
	HSF	94. 92 ± 1.41
	DCMSF	107.28 ± 1.45
	EASF	140.26 ± 0.03
	AQSF	168.99 ± 1.02

Data are presented as mean ± SD. BHT = tert-butyl-1-hydroxytoluene, ME = methanol extract, HSF = hexane soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, AQSF = aqueous soluble fraction of the roots of *B. ceiba*.

In this investigation, the AQSF showed strong free radical scavenging activity with an IC_{50} value of 3.33 ± 0.25 µg/ml, while the IC_{50} value of BHT was 61.90 ± 1.70 µg/ml. Other samples ME, HSF, DCMSF and EASF also exhibited antioxidant potential having IC_{50} value 10.25 ± 1.97, 62.47 ± 2.84, 20.80 ± 1.09 and 5.98 ± 2.34 µg/ml, respectively (Table 2).

Table 2. Free radical scavenging activity of the roots of *B. ceiba*.

Plant part	Sample code	IC_{50} (µg/ml) value
Root	BHT	61.90 ± 1.70
	ME	10.25 ± 1.97
	HSF	62.47 ± 2.84
	DCMSF	20.80 ± 1.09
	EASF	5.98 ± 2.34
	AQSF	3.33 ± 0.25

Data are presented as mean ± SD. BHT = tert-butyl-1-hydroxytoluene, ME = methanol extract, HSF = hexane soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, AQSF = aqueous soluble fraction of the roots of *B. ceiba*.

In brine shrimp lethality bioassay, the highest lethality was demonstrated by HSF having LC_{50} value of 1.19 ± 0.10 µg/ml as compared to 0.47 ± 0.01 for vincristine sulfate whereas ME, DCMSF and EASF showed moderate cytotoxicity but AQSF showed low activity (Table 3).

Table 3. Brine shrimp lethality bioassay of the root extract of *B. ceiba*.

Plant	Sample code	LC_{50} (µg/ml)
Root	ME	67.47 ± 13
	HSF	1.19 ± 0.10
	DCMSF	29.87 ± 11.17
	EASF	93.45 ± 4.95
	AQSF	890.57 ± 66.67
	VS	0.47 ± 0.01

Data are presented as mean ± SD. ME = methanol extract, HSF = hexane soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, AQSF = aqueous soluble fraction of the roots of *B. ceiba*.

The thrombolytic activity was studied for all the extractives of *B. ceiba* to evaluate its activity as a cardioprotective action. Among different fractionates of *B. ceiba*, the highest percentage of clot lysis 44.55 ± 0.12% was exhibited by AQSF (Table 4).

Table 4. Thrombolytic activity of the extractives of roots of *B. ceiba*.

Plant part	Sample code	% Clot lysis
Root	MESF	21.09 ± 1.42
	HESF	9.56 ± 1.21
	DCMSF	40.67 ± 0.47
	EASF	29.55 ± 0.22
	AQSF	44.55 ± 0.12
	Blank	3.80 ± 0. 34
	SK	64.22 ± 0.66

Data are presented as mean ± SD. ME = methanol extract, HSF = hexane soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, AQSF = aqueous soluble fraction of the roots of *B. ceiba*, SK = streptokinase.

The methanol extract of the roots of *B. ceiba* revealed significant anti-diarrheal activity in mice model at both doses of 200- and 400- mg/kg bw at the first, second, third and fourth hour (Table 5).

The crude methanol extract of *B. ceiba* revealed remarkable hypoglycemic activity in Swiss Albino mice at 200- and 400- mg/kg bw. After 180 minutes of administration, the crude methanol extract displayed 44.6% and 32.5% reduction of blood sugar level at 200- and 400- mg/ kg of body weight, respectively whereas the standard drug glibenclamide showed 50.36% reduction of blood glucose level in mice (Table 6).

The methanol extract of *B. ceiba* exhibited strong central analgesic activity in Swiss-Albino mice at both doses of 200- and 400- mg/kg bw after 30, 60 and 90 min in tail immersion method (Table 7).

The methanol extract of *B. ceiba* showed significant peripheral analgesic activity with percent inhibition of 45.12% and 62.76% at 200- and 400- mg/kg bw respectively (Table 8).

Table 5. Antidiarrheal activity of the crude methanol extract of *B. ceiba.*

Animal group	Number of diarrheal feces (average)							
	1 hr	% Reduction	2 hrs	% Reduction	3 hrs	% Reduction	4 hrs	% Reduction
CTL	1		3.0		2		2.33	
STD	0	100	0.67	77.67	0.67	66.5	0.67	71.24
ME 1	0.33	67	1.67	44.33	1	50	1	57.08
ME 2	0.33	67	0.67	77.67	1	50	1	57.08

ME = methanol extract of *B. ceiba*, STD = standard drug loperamide, CTL = control group (1%Tween 80 in normal sline).

Table 6. Hypoglycemic activity of the root of *Bombax ceiba.*

Animal group	0 Min	% Reduction	60 Min	% Reduction	120 Min	% Reduction	180 Min	% Reduction
CTL	5.13	-	13.40	-	9.50	-	8.40	-
STD	5.03	1.94	10.20	23.88	7.40	22.05	4.17	50.36
ME-1	4.80	6.43	11.30	15.67	6.63	30.21	4.67	44.40
ME-2	5.23	1.94	11.10	17.16	9.33	1.80	5.67	32.5

ME = methanol extract of *Bombax ceiba*, STD = standard drug glibenclamide (10 mg/kg bw), CTL = control group (1% Tween-80 & DMSO in normal saline 10 mg/kg bw).

Table 7. Central analgesic activity of the root extractives of *B. ceiba* in Swiss-Albino mice.

Sample		Average immersion time					
		After 30 min		After 60 min		After 90 min	
		(Average immersion time ± SD)	% Elongation	(Average immersion time ± SD)	% Elongation	(Average immersion time ± SD)	% Elongation
Root	CTL	2.16 ± 0.43		1.87 ± 0.18		2.23 ± 0.32	
	STD	6.18 ± 0.69	186.1	9.19 ± 0.34	391.44	13.15 ± 0.18	489.68
	ME 1	3.99 ± 0.12	84.72	7.15 ± 0.58	282.35	8.28 ± 1.53	271.30
	ME 2	3.62 ± 0.55	67.59	7.39 ± 0.73	295.19	9.94 ± 1.3	345.74

ME = methanol extract of *Bombax ceiba*, STD = standard drug diclofenac (50 mg/kg bw), CTL = control group (1% Tween-80 in normal saline 10 mg/ kg bw).

Table 8. Effect of methanol extract of the roots of *B. ceiba* on writhing test in mice.

Animal group	Number of writhing			Average ± SD	Inhibition (%)
	M-1	M-2	M-3		
CTL	16	18	17	17 ± 1	-
STD	4	5	4	4.33 ± 0.58	74.53
ME 1	9	11	8	9.33 ± 1.53	45.12
ME 2	6	6	7	6.33 ± 0.58	62.76

ME = methanol extract of *Bombax ceiba*, STD = standard drug diclofenac (50 mg/kg bw), CTL = control group (1% Tween-80 in normal saline 10 mg/kg bw), M-1,2,3 = mouse-1,2 and 3, respectively.

Conclusion

B. ceiba was found to be a potential plant for further chemical investigation, since it has significant antioxidant, brine shrimp lethality, thrombolytic, antidiarrheal, hypoglycemic, central and peripheral analgesic activities. The observed biological activities justify some of its traditional uses by the folk practitioner.

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