Studies of Bioactivities of *Adansonia digitata* (L.)

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ABSTRACT: The methanol extracts of leaves and barks of *Adansonia digitata* (L.) and their organic and aqueous soluble partitioning materials were evaluated for antioxidant, cytotoxic, thrombolytic, membrane stabilizing, antimicrobial, analgesic, and antidiarrhoel activities. In the DPPH free radical scavenging assay, the aqueous soluble materials of bark and leaves of *A. digitata* displayed the highest free radical scavenging activity with IC_{50} values of 2.21 µg/ml and 7.70 µg/ml, respectively while the crude extract of leaves and its pet ether soluble fraction showed significant lethality to brine shrimps having LC_{50} value of 6.99 and 0.284 µg/ml, respectively as compared to standard vincristine sulphate (LC_{50} value of 0.44 µg/ml). During assay for thrombolytic activity of human blood clot, the carbon tetrachloride soluble partitionate of leaves of *A. digitata* revealed 48.11% clot lysis. In the membrane stabilizing assay, the carbon tetrachloride- and aqueous-soluble fractions of methanol extract of leaves inhibited 61.52 % and 16.03 % hypotonic solution- and heat-induced haemolysis of RBCs, respectively. The methanolic extracts of leaves and barks of *A. digitata* revealed significant central and peripheral analgesic activity at 200 and 400 mg/kg body weight. The crude extract of leaves of *A. digitata* also displayed significant antidiarrhoel but mild antibacterial activities.

Key words: *Adansonia digitata*, antioxidant, DPPH, thrombolysis, antibacterial, analgesic, antidiarrhoel.

INTRODUCTION

A. digitata (L.) (English name: Monkey-bread tree; Bengali name: Baobab) belongs to the family Malvaceae. It is an emblematic, culturally important and physically majestic sub-tropical tree indigenous to Africa. Various parts of *A. digitata* are used as immuno-stimulant, anti-inflammatory, analgesic, insect repellent and pesticide, also in the treatment of diarrhea and dysentery.[1,2] Previous phytochemical investigations of *A. digitata* led to the isolation of terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids.[3,4]

As part of our continuing investigation on medicinal plants of Bangladesh[5,6], the crude methanol extracts of leaves and barks of *A. digitata* as well as their organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging, cytotoxic, thrombolytic, membrane stabilizing, antimicrobial, analgesic, and antidiarrhoel activities for the first time and we, here in, report the results of our preliminary investigations.

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MATERIALS AND METHODS

Collection of plant materials and extraction. The leaves and barks of *A. digitata* were collected in April, 2014. Voucher specimens (Accession no: 40130) for the plant have been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh for future reference.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 g each) from the plant 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 number 1 filter paper. The filtrates were concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of each of the concentrated methanol extracts was fractionated by the modified Kupchan partitioning protocol[7] and the resultant partitionates were evaporated to dryness to yield pet-ether (PESF), carbontetrachloride (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 *A. digitata* leaf and bark.

Crude extract/ Fractions*	Leaf (g)	Bark (g)
ME	5.00	5.00
PESF	0.75	0.65
CTCSF	0.65	0.50
CSF	0.40	0.30
AQSF	2.50	3.20

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

Drugs and chemicals. Acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), Morphine hydrochloride (Gonoshastho Pharmaceuticals), Diclofenac sodium, Glibenclamide and Loperamide were used in the investigation. The extractives of the plant were dissolved in 1 % Tween 80 and subsequently in 0.9 % normal saline separately at a concentration of 10 mg/ml and the required dose was administered according to the weight of each mice.

Animals. Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25g were used for the experiment. The procedures in this study for animal handling were performed in accordance with the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). All efforts were made to minimize animal sufferings and to reduce the number of animals used in the experiments. They were kept in standard environmental condition (at 24.0±1 °C temperature and 55-65 % relative humidity and 12 h light/12 h dark cycle) for a week for acclimation after their purchase and fed with rodent food purchased from ICDDR, B and water *ad libitum*.

Total phenolic content. The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd.[8]

DPPH free radical scavenging assay. Following the method developed by Brand-Williams[9], the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxy toluene (BHT) and ascorbic acid as reference standards.

Brine shrimp lethality bioassay. This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solution of plant extractives against *Artemia salina* in a single day assay by using vincristine sulphate as positive control.[10]

Thrombolytic activity. The method developed by Prasad and Harbertson[11] was used to determine the thombolytic activity by using streptokinase (SK) as positive control.

Membrane stabilizing activity. The membrane stabilizing activity of the extractives was evaluated by the inhibition of heat- and hypotonic solution-induced haemolysis of human erythrocytes following the method developed by Omale *et al*.[12]

Antimicrobial screening. Antimicrobial activity was determined by the disc diffusion method.[13]

Central analgesic activity. Evaluation of central analgesic activity was carried by tail immersion method using Morphine as a positive control. A constant heat stress was applied to rat tail, which acted as pain stimulus. When the stimulus exceeded the threshold, rat showed a quick withdrawal of its tail. Time taken by the rat to withdraw the tail is termed as tail immersion time. Analgesic compounds elongate this responding time which was recorded to observe central analgesic action.

Peripheral analgesic activity. Peripheral analgesic activity was evaluated by formalin-induced method.[14]

Antidiarrhoel activity. Antidiarrhoel activity was assessed by the castor oil-induced diarrhea in mice.[15]

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 leaves and bark of *A. digitata* as well as their Kupchan partitionates were evaluated for the total phenolic content, free radical scavenging, cytotoxic, thrombolytic, membrane stabilizing, antimicrobial, analgesic, and antidiarrhoel activities.

The total phenolic content of the extractives of leaves and barks of *A. digitata* were found in the ranges of 8.69 ± 0.23 to 17.75 ± 0.45 mg and 20.69 ± 0.27 to 70.27 ± 0.63 mg of GAE/g of extractives, respectively, with the highest amount of phenolics (17.75 ± 0.45 and 70.27 ± 0.63 mg) being observed in the methanol extract and pet ether soluble fraction, respectively (Table 2).

Table 2. Total phenolic content, free radical scavenging and cytotoxic activities of *A. digitata.*

Plant	Sample/ Standard	Total phenolic content (mg of GAE/gm of extract)	DPPH free radical scavenging activity (IC_{50} µg/ml)	Cytotoxicity (LC_{50} µg/ml)
Leaf	ME	17.75± 0.67	26.18± 0.43	0.348± 0.45
	PESF	16.06± 0.23	103.87± 0.23	0.284± 0.08
	CTCSF	13.44± 0.45	20.24± 0.76	15.65± 0.62
	CSF	10.28 ± 0.56	15.20 ± 0.65	11.8 ± 0.44
	AQSF	8.69± 0.33	7.70± 0.88	0.416± 0.59
Bark	ME	20.69± 0.63	5.63± 0.29	6.99± 0.76
	PESF	70.27± 0.27	62.22± 0.77	31.61± 0.23
	CTCSF	48.97± 0.46	2.40± 0.42	33.45± 0.63
	CSF	36.28 ± 0.55	13.53 ± 0.35	26.46 ± 0.54
	AQSF	30.83± 0.23	2.21± 0.47	13.0 ± 0.84
Standards	VS	-	-	0.44 ± 0.01
	BHT	-	27.70 ± 0.54	-
	Ascorbic acid	-	5.40 ± 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 hydroxy toluene; VS= Vincristine sulfate.

In the DPPH free radical scavenging assay, the aqueous soluble fraction of leaves of *A. digitata* revealed maximum free radical scavenging activity having IC_{50} value of 7.70 ± 0.88 µg/ml while the standard ascorbic acid showed IC_{50} value of 5.80 ± 0.21 µg/ml. Among the test samples of bark of *A. digitata*, the aqueous soluble materials demonstrated the highest free radical scavenging activity with IC_{50} value of 2.21 ± 0.29 µg/ml (Table 2).

In the brine shrimp lethality bioassay, the pet ether soluble partitionate of leaves of *A. digitata* displayed the highest cytotoxic potential with LC_{50} value 0.284 ± 0.08 µg/ml as compared to 0.44 ± 0.01 µg/ml for vincristine sulphate. On the other hand, the methanolic extract of barks of *A. digitata* revealed the highest cytotoxic potential with LC_{50} value 6.99 ± 0.84 µg/ml. This suggested the presence of potent bioactive components in the above mentioned extractives (Table 2).

The extractives of leaf and bark of *A. digitata* were assayed for thrombolytic activity to determine the ability of clot lysis. Standard streptokinase at 37 °C showed 65.88% lysis of the clot as compared to distilled water showing a negligible lysis of clot

(3.74%). In this study, the carbon tetrachloride soluble fraction of leaf of *A. digitata* showed highest thrombolytic activity (48.11 ± 0.88%) (Table 3).

Table 3. Thrombolytic activity of *A. digitata*.

Sample	% Clot lysis
	Leaf
ME	38.50± 0.55
PESF	37.13± 0.44
CTCSF	48.11± 0.34
CSF	45.11± 0.23
AQSF	46.76± 0.88
Blank	3.74 ± 0.55
SK	65.88 ± 1.08

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

The membrane stabilizing activity of the extractives of leaves of *A. digitata* was also determined. They significantly protected the lysis of human erythrocyte membrane-induced by both heat and hypotonic solution, as compared to the standard acetyl salicylic acid. In hypotonic solution-induced condition, the carbon tetrachloride soluble fraction of leaves of *A. digitata* inhibited 61.52 ± 0.84 % haemolysis of RBCs as compared to 72.2 % revealed by acetyl salicylic acid (0.10 mg/ml) (Table 4). In heat-induced condition, the aqueous soluble fraction of leaf of *A. digitata* inhibited 36.03 ± 0.21 % haemolysis of RBCs as compared to 42.2% showed by acetyl salicylic acid (0.10 mg/ml) (Table 4).

Table 4. Percent inhibition of hypotonic solution- and heat-induced hemolysis of erythrocyte membrane by leaf of *A. digitata*.

Sample	Hypnotic solution-induced	Heat-induced
ME	20.02± 0.66	29.68± 0.23
PESF	37.22± 0.55	8.82± 0.43
CTCSF	61.52± 0.84	32.27± 0.56
CSF	16.03± 0.67	2.15± 0.44
AQSF	16.03 ± 0.84	36.03± 0.32
Acetyl salicylic acid	72.2 ± 0.47	42.2 ± 0.23

Table 5. Effect of methanol extracts of *A. digitata* on tail immersion test in mice.

	Sample	**After 30 min**		**After 60 min**		**After 90 min**	
		average (immersion time count) ± SD	% elongation	average (immersion time count) ± SD	% elongation	average (immersion time count) ± SD	% elongation
Leaf	CTL	2.47 ± 0.23					
	STD	5.06 ± 0.44	104.89				
	ME 1	4.13 ± 0.32	67.21	4.55 ± 0.31	84.21	4.13 ± 0.23	67.21
	ME 2	4.54 ± 0.21	83.81	4.80 ± 0.45	94.32	4.54 ± 0.42	83.81
Bark	CTL	2.22 ± 0.23					
	STD	4.44 ±0.43	100.2				
	ME 1	3.51 ± 0.13	58.21	4.43 ± 0.76	99.54	4.15 ± 0.54	87.1
	ME 2	3.68 ± 0.76	65.75	4.08 ± 0.56	83.79	4.13 ± 0.23	86.1

ME 1 = methanolic crude extract at 200 mg/kg body weight CTL=control
ME 2 = methanolic crude extract at 400 mg/kg body weight STD= standard

The extractives of leaf of *A. digitata* when screened for antibacterial activity against gram positive and gram negative bacteria at a concentration of 400 µg/disc, the methanolic extract of leaves of *A. digitata* exhibited mild inhibition of growth of microorganisms. The inhibitory activity of the extractives was compared with ciprofloxacin as standard. The zone of inhibition produced was :

Bacillus cereus (8.0 mm), *B. megaterium* (10.0 mm), *B. subtilis* (8.0 mm), *Sarcina lutea* (9.0 mm), *Staphylococcus aureus* (11.0 mm), *Escherichia coli* (8.0 mm), *Salmonella* Paratyphi (8.0 mm), *S.* Typhi (10.0 mm), *Shigella boydii* (10.0 mm), *S. dysenteriae* (9.0 mm), *Pseudomonas aeruginosa* (7.0 mm), *Vibrio parahemolyticus* (8.0 mm), *Aspergillus niger* (11.0 mm), *Sacharomyces cerevisiae* (10.0 mm) as compared to ciprofloxacin (40-53 mm).

The methanol extract of leaves and barks of *A. digitata* showed significant central analgesic activity at both 200 and 400 mg/kg body weight after 30 min (Table 5).

Extractives of leaf showed statistically significant peripheral analgesic activity at both doses of 200 mg/kg and 400 mg/kg body weight with writhing inhibition of 21.1% and 30.5%, respectively. The bark extracts demonstrated significant peripheral analgesic activity also at 200 mg/kg and 400 mg/kg body weight with percent inhibition of writhing 52.2 % and 40.9 %, respectively (Table 6).

Table 6. Peripheral analgesic activity of methanolic crude extract of *A. digitata.*

Sample	Average writhing count ± SD	% Inhibition
Leaf		
CTL	15.67 ± 0.34	
STD	7.01 ± 0.43	55.3
ME 1	18.98 ± 0.21	21.1
ME 2	20.45 ± 0.13	30.5
Bark		
CTL	14.67 ± 0.34	
STD	6.01 ± 0.15	59.1
ME 1	7.01 ± 0.24	52.2
ME 2	8.67 ± 0.12	40.9

The methanolic extract of leaves of *A. digitata* showed significant anti-diarrheal activity at the first, second, third and fourth hour of administration of castor oil followed by the leaf extract (Table 7).

Table 7. Antidiarrhoel activity of leaf extractives of *A. digitata.*

Sample	1st hour		2nd hour		3rd hour		4th hour	
	Average no of faeces ± SD	% Inhibiton	Average no of faeces ± SD	% Inhibiton	Average no of faeces ± SD	% Inhibiton	Average no of faeces ± SD	% Inhibiton
CTL	4.33 ± 0.56	-	4.67 ± 0.14	-	6.01 ± 0.67	-	6.33 ± 0.67	-
STD	0.00 ± 0.00	100	0.33 ± 0.15	92.9	0.67 ± 0.33	88.8	0.33 ± 0.56	94.8
ME 1	1.33 ± 0.67	90.1	0.33 ± 0.18	92.9	1.67 ± 0.45	72.2	0.33 ± 0.45	94.8
ME 2	1.33 ± 0.67	90.1	0.21 ± 0.56	95.5	0.67 ± 0.13	88.8	0.67 ± 0.67	89.4

CONCLUSION

It is clearly evident from the above findings that the leaf and bark of *A. digitata* have significant free radical scavenging, cytotoxic, membrane stabilizing, thrombolytic, analgesic, and antidiarrhoel properties. The plant also exhibited mild antimicrobial potential. The leaf and bark of *A. digitata* have been reported to be used for inflammation, diarrhoea, pain, and other health disorders. Our findings justify some of the traditional uses of the plant species. Therefore, the plant is a good candidate for further chemical investigations to isolate the active constituents.

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