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Bioactivities of *Chukrasia tabularis* (A. Juss.)

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Abstract

The methanol extracts of bark and leaves of *Chukrasia tabularis* and their organic and aqueous soluble materials were subjected to screenings for antioxidant, cytotoxic, thrombolytic, membrane stabilizing, antimicrobial, analgesic and antidiarrhoel activities. In the DPPH free radical scavenging assay, the methanolic extract of bark of *C. tabularis* and the aqueous soluble fraction of methanol extract of leaves of *C. tabularis* revealed the highest free radical scavenging activity with IC_{50} values of 2.95 µg/ml and 5.31 µg/ml, respectively. The pet ether soluble fraction of methanolic extract of leaves and bark of *C. tabularis* displayed the highest cytotoxic potential having LC_{50} values 0.0167 µg/ml and 3.89 µg/ml, as compared to standard vincristine sulphate (LC_{50} value of 0.45 µg/ml). During thrombolytic assay, the aqueous soluble fraction of leaves and carbon tetrachloride soluble fraction of bark of *C. tabularis* showed 34.04% and 56.37% clot lysis, respectively. In the membrane stabilizing assay, the carbontetrachloride and aqueous soluble materials of methanol extract of leaf inhibited 21.03% and 49.68% hypotonic solution- and heat-induced haemolysis of RBC, respectively. The crude extract of leaves of *C. tabularis* exhibited mild antibacterial activity, while that of leaves and bark revealed significant central analgesic activity at 400 mg/kg body weight. The crude extracts demonstrated significant peripheral analgesic activity at 200- and 400-mg/kg body weight. On the other hand, the crude extract of leaves of *C. tabularis* revealed significant antidiarrhoel activity.

Key words*: Chukrasia tabularis*, antioxidant, DPPH, thrombolysis, antibacterial, analgesic, antidiarrhoel.

Introduction

Chukrasia tabularis (A. Juss.) (English name: Burmese almond wood; Bengali name: Chikrassi) belongs to the family Meliaceae. It is an evergreen deciduous, sometimes fairly large tree up to 30 m in height and is widely distributed in South East Asia including Bangladesh. Traditionally various parts of *C. tabularis* are used as analgesic, astringent, antipyretic, antidiarrhoeal activities. (Nakatani *et al*., 2004). Previous phytochemical investigations with *C. tabularis* led to the isolation of triterpenes, (Roy *et al*., 2006) and limonoids (Xhang *et al*., 2007).

As part of our ongoing investigation with medicinal plants of Bangladesh (Tahia *et al*., 2015, Sikder *et al*., 2011), the crude methanol extracts of the bark and leaves of *C. tabularis* as well as their aqueous and organic 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.

Materials and Methods

Collection of plant materials and extraction: The bark and leaves of *C. tabularis* were collected in April 2014. Voucher specimens (Accession no: DACB-40132) 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 bark and leaves in powdered form (500 g each) were separately soaked in 2.0 liters of

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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 g) of each of the concentrated methanol extracts was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) and the resultant partitionates were evaporated to dryness 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.

Drugs and chemicals: Acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), Morphine (Gonoshastho Pharmaceuticals), Diclofenac sodium, Glibenclamide and loperamide were used in this 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 orally according to the weight of the mice.

Table 1. Kupchan partitionates of *C. tabularis* leaf and bark.

Crude extract/ Fractions	Leaf (g)	Bark (g)
ME	5.00	5.00
PESF	0.65	0.85
CTCSF	0.55	0.75
CSF	0.30	0.40
AQSF	2.50	2.30

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

Animals: Swiss-albino mice of either sex aged 4–5 weeks were used for the experiment. The average weight of the mice used was 20-25 g. 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 animals suffering 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 feed purchased from International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B) and water.

Total phenolic content: The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent by using the method developed by Skerget *et. al.*, (2005).

DPPH free radical scavenging assay: Following the method developed by Brand-Williams *et al.*, (1995), 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 hydroxytoluene (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 (Meyer *et al*., 1982). Vincristine sulphate was used as the positive control.

Thrombolytic activity: The method developed by Prasad *et al.*, (2006) and Harbertson *et al*., (2006) 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*., (2008).

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

Tail immersion technique for peripheral analgesic assay: The tail immersion method was carried out as an alternative method to evaluate peripheral analgesic activity (Aydin *et al*., 1999).

Antidiarrheal activity: Antidiarrhoeal activity was assessed by the method developed by Shoba and Thomas, 2001. Castor oil was used to induce diarrhea.

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 bark and leaves of *C. tabularis*, 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 leaves of *C. tabularis* was found in the range of 3.91 ± 0.23 to 21.07 ± 0.45 mg of GAE/g of extractives, with the highest amount of phenolics (21.07 ± 0.45 mg) was observed in the carbon tetrachloride soluble fraction. Again, the total phenolic content of the bark of *C. tabularis* was found in the range of 25.21 ± 0.27 to 139.53 ± 0.63 mg of GAE/g of extractives, with the highest amount of phenolics (139.53 ± 0.63 mg) being observed in the methanol extract (Table 2).

In the DPPH free radical scavenging assay, the aqueous soluble fraction of leaves of *C. tabularis* revealed maximum free radical scavenging activity having IC_{50} value of 5.31 ± 0.88 µg/ml while standard ascorbic acid showed IC_{50} value of 5.80 ± 0.21 µg/ml. Among the test samples of bark of *C. tabularis*, the methanol extract demonstrated the highest free radical scavenging activity with IC_{50} value of 2.95 ± 0.29 µg/ml (Table 2).

Table 2. Total phenolic content, free radical scavenging and cytotoxic activities of *C. tabularis*.

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	14.00 ± 0.67	7.39 ± 0.43	12.912 ± 0.45
	PESF	3.91± 0.23	43.08 ± 0.23	0.0167 ± 0.08
	CTCSF	21.07 ± 0.45	20.70 ± 0.76	10.27 ± 0.62
	CSF	10.28 ± 0.56	15.20 ± 0.65	21.8 ± 0.44
	AQSF	6.84 ± 0.33	5.31 ± 0.88	4.36 ± 0.59
Bark	ME	139.53 ± 0.63	2.95 ± 0.29	13.8 ± 0.76
	PESF	25.21 ± 0.27	21.40 ± 0.77	10.05 ± 0.23
	CTCSF	66.60 ± 0.46	8.81 ± 0.42	4.46 ± 0.63
	CSF	76.28 ± 0.55	13.53 ± 0.35	36.46 ± 0.54
	AQSF	131.81 ± 0.23	410.72 ± 0.47	3.89 ± 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 hydroxytoluene; VS= Vincristine sulfate.

In the brine shrimp lethality bioassay, the pet ether soluble fraction of leaves of *C. tabularis* displayed the highest cytotoxic potential with LC_{50} value 0.0167 ± 0.08 µg/ml as compared to 0.44 ± 0.01 µg/ml for vincristine sulphate. On the other hand, the pet ether soluble fraction of bark of *C. tabularis*, revealed the highest cytotoxic potential with LC_{50} value 3.89 ± 0.84 µg/ml. This suggested the presence of potent bioactive components in the above mentioned extractives (Table 2).

The extractives of the bark and leaves of *C. tabularis* were assayed for thrombolytic activity to determine the ability to lyse clots. Upon 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, showed 65.88% lysis of the clot as compared to distilled water showing a negligible lysis of clot (3.74%). In this study, the aqueous soluble fraction of leaf of *C. tabularis* showed highest thrombolytic activity of 56.37 ± 0.88% while the carbon tetra chloride soluble fraction of bark of *C. tabularis* showed maximum activity of 34.04 ± 0.23% (Table 3).

The membrane stabilizing activity of the extractives of the leaves of *C. tabularis* was also determined. They significantly protected the lysis of human erythrocyte membrane induced by heat and hypotonic solution, as compared to the standard acetyl salicylic acid. In hypotonic solution- and heat-induced conditions, the carbon tetrachloride and aqueous soluble fractions of leaves of *C. tabularis* inhibited haemolysis of RBCs by 21.03 ± 0.84% and 49.68 ± 0.21% as compared to 72.2% and 42.2% revealed by acetyl salicylic acid (ASA, 0.10 mg/ml), respectively (Table 4).

Table 3. Thrombolytic activity of *C. tabularis.*

Sample	% clot lysis	
	Leaf	bark
ME	37.68 ± 0.55	5.89 ± 0.33
PESF	23.89 ± 0.44	21.63 ± 0.23
CTCSF	29.99 ± 0.34	11.93 ± 0.11
CSF	41.78 ± 0.23	34.04 ± 0.23
AQSF	56.37 ± 0.88	9.44 ± 0.56
Blank	3.74 ± 0.55	
SK	65.88 ± 1.08	

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

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

Sample	Leaf	
	Hypnotic solution induced	Heat-induced
ME	14.34 ± 0.66	49.68 ± 0.23
PESE	0.90 ± 0.55	8.82 ± 0.43
CTCSF	21.03 ± 0.84	32.27 ± 0.56
CSF	1.47 ± 0.67	2.15 ± 0.44
AQSF	57.4 ± 0.84	49.68 ± 0.32
ASA	72.2 ± 0.47	42.2 ± 0.23

The extractives of the leaves of *C. tabularis* were screened for antibacterial activity against gram positive and gram negative bacteria at a concentration of 400 µg/ disc. The methanolic extract of the leaves of *C. tabularis* exhibited mild antimicrobial activity. The inhibitory activity of the extractives was compared with ciprofloxacin as standard (Table 5)

The methanol extract of the leaves of *C. tabularis* showed significant peripheral analgesic activity at 400 mg/kg body weight after 30 minutes of administration. Both 200- and 400-mg/kg body weight showed significant central analgesic effect after 60 and 90 minutes respectively. The methanol extract of the bark of *C. tabularis* revealed significant activity after 30 minutes at 400 mg/kg body weight (Table 6).

The methanolic extract of leaves of *C. tabularis* showed significant anti-diarrheal activity at the first, second, third and fourth hour (Table 7)

Table 5. Antibacterial activity of leaf of *C. tabularis.*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTCSF	CSF	AQSF	Ciprofloxacin
Bacillus cereus	9.0					40.0
B. megaterium	11.0					
B. subtilis	9.0					40.0
Sarcina lutea	10.0					50.0
Staphylococcus aureus	10.0					53.0
Escherichia coli	9.0					47.0
Salmonella Paratyphi	10.0					51.0
S. Typhi	100				10.0	49.0
Shigella boydii	11.0	9.0				47.0
Sh. dysenteriae	10.0					47.0
Pseudomonas aeruginosa	10.0					45.0
Vibrio mimicus						45.0
V. parahaemolyticus	9.0					48.0
Aspergillus niger	13.0					47.0
Candida albicans	8.0					45.0
Sacharomyces cerevisiae	12.0			8.0		45.0

Table 6. Effect of methanol extracts of *C. tabularis* on tail immersion test in mice.

	Sample	After 30 min (Average immersion time ± SD)	% Elongation	After 60 min (Average immersion time ± SD)	% Elongation	After 90 min (Av.immersion time ± SD)	% Elongation
Leaf	CTL	2.68 ± 0.23					
	STD	5.762 ± 0.44	115				
	ME 1	3.44 ± 0.32	28.36	5.30 ± 0.31	98.65	5.55 ± 0.23	107
	ME 2	3.85 ± 0.21	43.65	5.55 ± 0.45	107.43	5.56 ± 0.42	107
Bark	CTL	2.19 ± 0.23					
	STD	6.48 ±0.43	196				
	ME 1	3.465 ± 0.13	58.21	4.31 ± 0.76	99.54	5.19 ± 0.54	137
	ME 2	3.63 ± 0.76	65.75	5.05 ± 0.56	133.79	5.17 ± 0.23	136

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

Table 7. Antidiarrheal activity of leaf extracts of *C. tabularis.*

Sample	1st hour		2nd hour		3rd hour		4th hour	
	(Ave no. of faeces ± SD)	% Inhibiton	(Ave no. of faeces ± SD)	% Inhibiton	(Ave no. of faeces ± SD)	% Inhibiton	(Ave no. of faeces ± SD)	% Inhibiton
CTL	2.66 ± 0.56	-	4.33 ± 0.14	-	6.33 ± 0.67	-	7.33 ± 0.67	-
STD	0.00 ± 0.22	100	0.00 ± 0.15	100	0.33 ± 0.33	94.8	0.66 ±0.56	90.9
ME 1	2.66 ± 0.67	0	2.66 ± 0.18	38	0.66 ±0.45	89.5	1.33 ±0.45	81.9
ME 2	1.33 ±0.14	50	0.66 ±0.56	84.8	1.00 ± 0.13	84.1	1.33 ±0.67	81.9

Conclusion

It is clearly evident from the above findings that the bark and leaves of *C. tabularis* have significant free radical scavenging, cytotoxic, membrane stabilizing, thrombolytic, central and peripheral analgesic, and antidiarrhel properties. The plant also exhibited mild antimicrobial potential. The bark and leaves of *C. tabularis* can be used for inflammation, pain etc. Our findings justify 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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