

## Anthelmintic and diuretic activity of bark extracts of *Sterculia villosa*

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### ABSTRACT

The study was evaluated for diuretic and anthelmintic activity of the ethanolic extract of the barks of *Sterculia villosa* Roxb. (Sterculiaceae). The diuretic assay was done on both healthy wistar rats and rabbits. The dose used for the diuretic assay was 100, 200 & 400mg/kg of the extract. Compared to the control and standard drug furosemide (20mg/kg), the result of diuretic study showed dose dependent activity of the extracts. The result also indicated higher excretion of Na<sup>+</sup>, K<sup>+</sup> & Cl<sup>-</sup> in urine. *Pheretima posthuma*, adult earthworms were used for anthelmintic activity and the results of anthelmintic assessment at the dose of 50, 100 & 200mg/ml showed significant activity compared with control and standard drug albendazole (10mg/ml).

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### INTRODUCTION

*Sterculia villosa* Roxb. (Sterculiaceae) are deciduous tree with grey or brown bark covered with corky nodules commonly known as 'Udal' or 'Udar'. It is one of the fast-growing plant species, has large long-stalked deeply lobed leaves and yellow flowers (Joshi, 2011). It is distributed More or less throughout North Eastern region of India, Bangladesh, Sri Lanka, Southern China (Ghani, 2003). Some chemical constituents like flavonoids, chrysoeriol, diosmetin-7-O-β-D-glucoside and chrysoeriol-7-O-β-D-glucoside were isolated from *S. villosa* (Seetharaman, 1990).

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The plant is used by Indians for traditional remedy of Inflammation (Namsa *et al.*, 2009). The plant also has antioxidant activity (Kshirsagar and Upadhyay, 2009). Traditionally, the plant is used in diuretic, cooling and aphrodisiac properties (Kumar *et al.*, 2004). Our study was designed to rationalize the traditional use of *S. villosa* by evaluating the diuretic effects on healthy rats and rabbits. Generally, diuretics are the compounds which promote the flow of urine in larger amounts than the normal. The diuretics are particularly useful in the treatment of ascites, congestive cardiac failure, and edema of the lungs, kidneys and liver. In these conditions fluid abnormally accumulates in the body. Diuretics increase the excretion of Na<sup>+</sup> which may induce antihypertensive effect. Such compounds may act on nephron in the kidney leading to a decrease in the re-absorption of water and subsequently increased urine output (Singh and Dixit, 1992).

They are commonly used for management of hypertension and electrolytic balance. So, the present study assesses the diuretic effects of the ethanolic extract of *S. villosa* barks.

Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas (Dhanvantari *et al.*, 1989). Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Anthelmintic drugs are used to destroy or cause the expulsion of such parasitic intestinal worms and helps to treat helminthiasis, one of the most common infections in humans and cattle. Albendazole is the first drug of choice for the treatment of worm infections. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Sondhi *et al.*, 1994). Hence there is an increasing demand towards natural anthelmintics. To overcome the resistance, plant derived drugs can serve as prototype to develop more effective and less toxic medicines (Rastogi *et al.*, 2009). The modern system of medicine claims that plants can be the cheapest source of treatment and can be safely used only after safety evaluation and dose determination for its effective use. Once the safety of plant and its dose dependent effectiveness is determined, plants can serve humans to treat and heal almost all the ailments. However, increasing problems of development of resistance in helminthes (Dorny *et al.*, 1995) have led to the proposal of screening medicinal plants for their anthelmintic activity. The purpose of this study was to evaluate ethanolic extract of *S. villosa* barks as new potential sources of natural anthelmintic.

## MATERIALS AND METHODS

### Plant material

*Sterculia villosa* Roxb, (Sterculiaceae) fresh bark collected from Rangamati, Bangladesh in september 2011 were authenticated by the taxonomist Dr. Jasim Uddin, Associate Professor, Department of Botany, University of Dhaka and the identification number was documented as Accession no DUSH 6905 and Call no 01.

### Preparation of extracts

The barks of *Sterculia villosa* washed with distilled water to remove dirt and soil. Then the barks were cut into small pieces and then dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted thrice with ethanol. The extracts were filtered, pooled and concentrated under reduced temperature on a rotary evaporator. The extract was stored in a refrigerator and used for the present study.

### Drugs and chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade. Albendazole was collected from Beximco Pharmaceuticals Ltd., Bangladesh. DMF (Merck, Germany) was used.

### Test animals: Earthworms

Adult earthworms (*Pheretima posthuma*) were collected from moist soil of Savar area of Dhaka in Bangladesh and washed with normal saline to remove all fecal matters. Then the worms were used for anthelmintic study. The earthworms of 8-10 cm in lengths and 0.3-0.5 cm in width were used for all the experimental protocol.

### Test animals: Rats

Albino (Wistar) rats 150-200 gm of either sex were used for the study. The animals were kept in the standard polypropylene cages and provided with food and water ad libitum. The animals were housed under standard environmental conditions with controlled conditions of temperature ( $23 \pm 2$  °C), humidity ( $50 \pm 5$  %) and 12 hours light-dark cycles. The animals were acclimatized for a period of 14 days prior to perform the pharmacological experiments.

### Test animals: Rabbits

The study was conducted on 25 healthy rabbits of either sex weighing between 2.5 to 3.5 kg. Animals were housed in separate cages under controlled conditions of temperature ( $23 \pm 2$  °C) and humidity ( $50 \pm 5$  %). All animals were given green leafy diet and water regularly. The experiments were performed according to the current guidelines for the care of the laboratory animals.

### Assessment of anthelmintic activity

The anthelmintic activity was evaluated on adult earthworm *Pheretima posthuma* (Ajaiyeoba *et al.*, 2001; Deore *et al.*, 2009). All worms were divided into five groups (Six worms in each group) and used to assess the anthelmintic properties of ethanolic extracts of *S. villosa* barks. Group I was the control, worms placed in normal saline and groups II, III & IV were treated with 50, 100 & 200mg/ml of ethanolic extracts *S. villosa* barks respectively. In order to administer these doses, the extracts were measured respectively and were dissolved in minimum amount of DMF. After proper mixing of extract and DMF, normal saline was slowly added. Group V received albendazole at the dose of 10mg/ml. It was dissolved in minimum amount of DMF and finally the volume was adjusted with normal saline. The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*, 2001 with minor modifications (Ajaiyeoba *et al.*, 2001). Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C), followed with their body colors fading away.

Due to the anatomical and physiological resemblance to the human intestinal round worm parasite, the assay was performed on adult earthworm, *Pheretima posthuma* (Vidyarthi, 1967; Chatterjee, 1967). The individual earthworms were placed in 50ml of five different formulations containing normal saline, three

different concentrations of crude ethanol extract (50mg/ml, 100mg/ml and 200mg/ml in saline water) and standard Albendazole (10mg/ml) suspension. The Time for paralysis and time for death was noted. The earthworms in control (group I) were alive up to 24 hours of the experiment.

### Assessment of Diuretic Activity on Rats

Male Wistar rats weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz *et al.* (1943) and Murugesan *et al.*, (2000) was employed for the assessment of diuretic activity. Five groups of six rats in each group were fasted and deprived of water for 18 hours prior to the experiment. All the animals received priming dose of normal saline solution of 25ml per kg body weight. First group (negative control) received 5ml/kg of body weight of normal saline, second group (positive control) received Furosemide (20mg/kg) and the third, fourth & fifth groups received the ethanolic extract in normal saline at the doses of 100, 200 & 400mg/Kg respectively. Immediately after administration, the rats (one in each cage) were placed in metabolic cages specially designed to separate urine and feces and kept at room temperature of 25±0.5°C throughout the experiment. The urine was collected in measuring cylinders up to 5 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for each individual rat was total urine volume, concentration of Na<sup>+</sup>, K<sup>+</sup> & Cl<sup>-</sup> in urine. Na<sup>+</sup>, K<sup>+</sup> concentrations were measured by Flame photometry (Jeffery *et al.*, 1989) and Cl<sup>-</sup> concentration was estimated by titration (Beckette and Stenlake, 1997) with silver nitrate solution(N/50)using three drop of 5% potassium chromate solution as indicator. The mean urine volumes were determined and diuretic potency was assessed by comparison of urine excretion due to the extracts with respect to the standard drug furosemide.

### Assessment of Diuretic Activity on Rabbits

We also took Male white rabbits that were again divided into five groups for the same test. Normal saline, furosemide and ethanolic extract (100mg/kg, 200mg/kg, 400mg/kg) are administered in the same way as in male rat. After 20 minutes the rabbits were intravenously anaesthetized with 3% (w/v) sodium pentobarbital solution at 30 mg/kg body weight. Sterile liquid paraffin was used to lubricate the catheter that was inserted in the bladder to discharge urine from all groups after a brief period of fasting.

**Table 2:** Diuretic activity of *S. villosa* barks in rats.

Treatment	Dose (mg/kg)	Urine Volume (ml)	Electrolyte Excretion			Na+/K+ ratio	Diuretic index	Lipschitz value	% Increase in Urine
			Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)				
Control	25ml/kg	4.391±0.078	90.8±0.77	51.64±2.18	96.85±1.12	1.78	-	-	100
Furosemide	20	10.278±0.095	138.4±1.78	95.75±1.39	159.61±1.10	1.44	2.34	-	59.91
Ethanolic Extract	100	6.158±0.089	101.5±1.47	69.15±1.17	119.44±1.46	1.51	1.40	0.60	66.37
Ethanolic Extract	200	6.822±0.092	112.2±1.36	74.26±1.12	125.58±1.31	1.41	1.58	0.66	79.56
Ethanolic Extract	400	8.178±0.109	121.9±1.37	86.38±1.21	141.37±1.38	1.41	1.55	0.79	

Furosemide urine output taken as 100% activity. Values are Mean±SEM, p<0.05. Diuretic Index=Mean urine volume of test/Mean urine volume of control. Lipschitz value = Mean urine volume of test/Mean urine volume of standard.

Again physiological saline (0.9% NaCl) was supplied to the animals at a speed of 2ml/min by ear vein injection at 10 ml/kg body weight. Catheter was used to collect the urine that was measured up to 4 hours of treatment (Xu *et al.*, 2002).

### Statistical Analysis

The results are expressed as Mean values ± S.E.M. (standard error of mean). Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Bonferroni Multiple Comparisons. The results were considered statistically significant when P < 0.05.

## RESULTS AND DISCUSSION

### Anthelmintic Activity

In this anthelmintic assay, extract of *S. villosa* barks demonstrated paralysis as well as death of worms. As shown in Table 1, ethanolic extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 200mg/ml concentration. Higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. Evaluation of anthelmintic activity was compared with reference standard drug Albendazole.

The ethanolic extract of *Sterculia villosa* barks were found to be effective upon comparison with the Standard drug albendazole in causing paralysis and death of earthworms. It can be concluded that active constituents responsible for anthelmintic activity might be present in the bark extracts.

**Table 1:** Anthelmintic activity of ethanolic extracts of *S. villosa* barks.

Sl No.	Test Group	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Control	-	-	-
2	Ethanol Extracts	50	69.24±1.75	81.42±2.08
3	Ethanol Extracts	100	63.51±1.79	76.58±2.35
4	Ethanol Extracts	200	54.38±2.39	69.39±3.27
5	Albendazole	10	26.43±1.94	43.64±2.13

All values represent Mean±SE; p<0.05.

### Diuretic Activity in Rats

The present study revealed that, ethanolic extract of *S. villosa* barks significantly increased the urinary output as well as urinary electrolyte concentration in normal rats.

**Table 3:** Diuretic Activity of *S. villosa* barks in Rabbits.

Treatment	Dose (mg/kg)	Urine Volume (ml)	% Increase in Urine
Control	Normal Saline	19.49±0.34	-
Furosemide	20	58.68±0.51	100
Ethanollic Extract	100	29.51±0.59	50.28
Ethanollic Extract	200	35.82±0.72	61.04
Ethanollic Extract	400	43.67±0.69	74.42

Furosemide urine output taken as 100% activity. Values are Mean ± SEM,  $p < 0.05$ .

Urine output of ethanolic extracts treated animals was more than saline treated animals. However the urine output of treated animals was less than standard drug treated animals. Maximum urine output was obtained at the dose of 400mg/kg of ethanolic extract treated animals. Maximum Sodium, Potassium & chloride concentration was also observed in 400mg/kg dosed ethanolic extract treated animals. These features suggest that the plant extract is acting in a similar way as furosemide, which increases urinary output and urinary excretion of sodium by inhibiting Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> transporter system in the thick ascending loop of Henley (Jackson, 1996).

#### Diuretic Activity in Rabbits

Diuretic effects of ethanolic extracts of *S. villosa* barks on male rabbits are shown in table-3. The result of diuretic activity of the present study showed that the ethanolic extract of *S. villosa* barks at 100, 200 and 400 mg/kg caused a dose dependent increase of urinary water in rabbits. The results of 400mg/kg treated group showed maximum diuretic effects and urine volume ( $P \leq 0.01$ ) compared with control group.

#### CONCLUSION

From the results of the present study, it is concluded that *S. villosa* showed significant anthelmintic activity. The plant may be further explored for its phytochemical profile to recognize the active constituent responsible for anthelmintic activity. As diuretics lessen pulmonary congestion and peripheral edema, these agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They increase plasma volume and subsequently venous return to the heart. This decreases cardiac work load, oxygen demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients. On the basis of the results of the present investigations, we can conclude that the ethanolic extracts of *S. villosa* barks found to produce significant diuretic activity, in a dose dependent manner up to 5 hours of drug administration by increasing the total urine output and increased excretion of sodium and potassium salts in male wistar rats. It also showed similar diuretic activity in healthy rabbits.

Hence, further isolation of active components will be advantageous to identify novel bioactive compounds from the extracts which may possess more significant activity.

**Conflict of interest:** The authors declare no conflict of interest.

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