

Research Article

Evaluation of Diuretic Activity of Acetone and Ethanol Stem Barks Extracts of *Spondias pinnata* (Linn.F) Kurz in Rats

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ABSTRACT

In this study, acetone and ethanol extracts of *Spondias pinnata* (Linn.F) Kurz stem barks were tested for Diuretic activity (Lipschitz test) in Wister albino rats. Furosemide (10mg/kg,p.o) used as reference standards respectively for activity comparison. The ethanolic extract posses significant diuretic activity. On the other hand, the acetone extract did not reveal significant activity. Urinary level of sodium, potassium (by flame photometry) and chloride (by titrimetry) were estimated.

Key words: Diuretic; *Spondias pinnata* (Linn.F) Kurz ; Acetone extract; Ethanolic extract.

INTRODUCTION

As presently available synthetic diuretic drugs pose several health problems during their clinical use, search to develop new and more effective drugs with fewer side effects is necessary. The use of natural products is growing in the world especially in developing country like India, where over 75% of the population relies mainly on plants and plant extracts for health care.

Spondias pinnata (Linn.F) Kurz is found in tribal areas of Mayurbhanj district and extensively used traditionally by the tribal people as anthelmintic, anti-inflammatory, anti-pyretic, anti-tumour and anti-bacterial activity¹⁻⁶. Plant also called Indian hog-plum (English), amara (Hindi), ambalam (Tamil), avimamadi (Telgu), ambula (Oriya). It is a glabrous tree 9-10.5 mtr. high; trunk straight; bark smooth, ash-coloured; branches nearly horizontal. Leaves 30-45 cm long, the common petioles slender, terete, smooth, striate, leaflets 3-5 pairs and a terminal one 7.5-18 by 3.8-7.5 cm oblong or elliptic – oblong, acuminate, quite entire, more or less oblique, main nerves numerous, horizontal, straight joined by a strong intra marginal one, petiolules 5-6 mm long. Flowers 1 – or 2 – sexual, sessile, numerous, pinkish green, in sparingly branched glabrous terminal panicles 25-38cm long. Calyx – teeth minute, triangular. Petals 2.5 – 3mm long, ovate- oblong, acute disk 10 – crenate. Stamens 10, about half as long as the petals. Drupes ovoid, yellow, about 3.8cm

long; stone woody, hard, rough with irregular furrows and cavities, fibrous outsides. Seeds usually, more rarely 2 or 3⁷.

The plant is reported to contain β -Amyrin and oeanolic acid, glycine, cystine, serine, alanine and leucine in fruits. Lignoseric acid, β -sitosterol and its glucoside in aerial parts⁸.

The literature subject reveals that various part of *Spondias pinnata* (Linn.F) Kurz have used as folklore medicine for curing various ailments like dysentery and diarrhoea, rheumatism, vomiting (bark); regulating mensuration (roots); anti-tubercular (plant); flavouring agent, dysentery(leaves); aphrodisiac (unripe fruits); constipation and anti-scorbutic(ripe fruits)⁹.

On this basis, the present study was under taken to report the diuretic activities of the stem barks of acetone and ethanol extract of *Spondias pinnata*.

MATERIALS AND METHODS

Plant Materials

The bark of *Spondias pinnata* (Linn.F) kurz (Anacardiaceae) was collected from young matured plants at the rural belt of Mayurbhanj district in the month of sept-2007 and was authenticated by taxonomist of botanical survey of India, Shibpur, Howrah, West Bengal (Letter

No.CNH/II/(177)/2007/Tech.II/113,dt.12-07-2007), Kolkata and Voucher Specimen was deposited there. The bark was shade –dried, pulverized in a mechanical grinder and stored

in a room temperature in a closed container for further use.

Preparation of extracts

The powdered plant materials (350 gm) was repeatedly extracted in a 2000 ml round bottomed flask with 1500 ml solvents of increasing polarity starting with petroleum ether, acetone and ethanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature and filtered.

On evaporation of acetone and ethanol under reduced pressure, a dark brown colored residue was obtained and the percentage yield is 4.69 % w/w, 11.67% w/w respectively and was stored in desiccators. For pharmacological experiments a weighed amount of the dried extract was dissolved in normal saline.

Phytochemical Screening

The extract was subjected to qualitative chemical investigation for the identification of different phyto constituents like carbohydrate, glycoside, proteins, fixed oil, alkaloid, saponin, flavonoids, phytosterol, phenolic compound and tri-terpenoids^{10, 11}.

The preliminary phyto chemical studies of acetone and ethanol extracts of *Spondias pinnata* stem bark were shown in Table 1. Which indicate the presence of carbohydrate, glycoside, saponin, flavonoids, phenolic compounds, phytosterols and tri-terpenoids.

Animals

Wister Albino rats of both sex weighing (100-200) gram were used for the evaluation of anti-inflammatory activity. The animals were maintained on the suitable nutritional and environmental conditions throughout the experiment as per the rules and regulations of the Institutional Animal Ethics Committee, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, Odisha. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional Animal Ethical Committee (Vide approval No. A5/12/IAEC/SIPS).

Toxicity studies

An acute toxicity study was performed to determine LD₅₀ using different doses of both the extracts according to the method described under CPCSEA guidelines. The animals were divided into different groups of ten animals each. The control group received 10 ml/kg body weight of 0.5% v/v tween 80 in distilled water orally. The other groups received the extracts of *Spondias pinnata* at a

dose level of 100-2000 mg/kg body weight through oral route. After administration of dose the animals were observed continuously for the first 4 hr for toxic symptoms like motor activity, tremors, convulsions, tonic extension, muscle spasm, loss of righting reflex, ataxia, sedation, diarrhea, salivation, writhing, skin colour and for mortality, if any, at the end of 24, 48 and 72 hr¹².

In acute toxicity study, the acetone and ethanol extracts of *S.pinnata* stem bark did not shown lethality up to the dose level of 2000 mg/kg, which indicates as a safe drug.

Diuretic activity in rats (Lipschitz test)

The diuretic activity of the test compound were evaluated in wistar rats weighing between 100-200 gm employed for this method^{13,14,15}. Group-1 served as control and fed with 0.5% v/v Tween 80 in normal saline orally at 10 ml/kg body weight. Group-2 received furosemide (10 mg/kg body weight) orally and served as standard. Group-3 to 5 were administered with acetone extracts of stem bark and group-6 to 8 were administered with ethanol extracts of stem bark respectively at a dose of 100, 200 and 400mg/kg body weight (p. o.) in a similar manner. Immediately after administration of the drug, the rats (3 in each cage) were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless steel sieves were placed in the funnel to retain feces and to allow the urine to pass. During the period of experiment, no food and water was provided to the animals. The total volume of urine excreted by the animals was collected after 5 hr and 24 hr of administration. Urine volume excreted per 100 gm body weight was calculated for each group. Urine samples were analyzed thereafter for Na⁺, K⁺ and Cl⁻. The concentration of Na⁺ and K⁺ were analyzed by flame photometer and the amount of Cl⁻ was determined titrimetrically by silver nitrate solution using one drop of 5% potassium chromate solution as indicator¹⁶.

Statistical analysis

The data were analyzed for significance by using the unpaired two-tailed student's *t*-test. $P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ was considered significant difference between control and others groups and there is no significance difference between standard and test drug at $^{#}P < 0.05$ significant level in all experiments. All other data was analyzed with simple statistics. The simple statistical analysis and paired samples *t*-test were conducted using Med Calc software version 11.6.1.0.

RESULTS AND DISCUSSION

Furosemide treated rats showed a significant increase in volume of urine and urinary excretion of sodium, potassium, and chloride ($p < 0.001$) as compared to control. Higher electrolyte (sodium and potassium) excretion ($p < 0.001$) was observed in rats treated with ethanol stem bark extract at a dose of 200 mg/kg and 400 mg/kg body weight as compared with the control group. The acetone stem bark extract at a dose of 400mg/kg produces significant amount of sodium and potassium ($P < 0.05$) respectively within 24 hr. At the dose of ethanol 100 mg/kg produces a significant amount of sodium and potassium ($P < 0.01$) within 24 hr. There was no significance difference in amount of electrolyte excretion between ethanol stem bark extract at a dose of 400 mg/kg body weight and standard drug at a level of ($P < 0.05$).

Consequently, significant increase in urine output ($P < 0.001$) were observed in ethanol extract at the dose level of 200 mg/kg and 400 mg/kg body weight after 5 hr and 24 hr. The ethanol stem bark extract at a dose of 100 mg/kg showed significantly ($P < 0.001$) and ($P < 0.05$) increases the urine output after 5 hr and 24 hr as well as compared with the control group.

The ethanol stem bark extract of *Spondias pinnata* at a dose of 200 mg/kg and 400 mg/kg produces significant diuretic activity which was comparable with the standard drug furosemide at a level of ($P < 0.05$). The results have been summarized in Table 2 and Figure 1.

CONCLUSION

Evaluation of diuretic activity of *Spondias pinnata* stem bark extracts by Lipschitz test. The ethanol extracts of *Spondias pinnata* at a dose of 200 mg/kg and 400 mg/kg are significantly ($p < 0.001$) increases the urine excretion after 5 hr and 24 hr of administration as compared with the control group. The result of the experiment revealed that the diuretic activity of the ethanol stem bark extract at a dose of 200 mg/Kg and 400 mg/Kg body

weight are comparable to that of reference standard furosemide at a dose of 10 mg/Kg body weight. It has been demonstrated that ethanol stem bark extract of *Spondias pinnata* produced diuretic effect by increasing the excretion of Na^+ , K^+ and Cl^- ions. Hence the ethanol stem bark extract is having significant diuretic activity as comparable with furosemide (standard).

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles¹⁷. The regulation of Na^+ / K^+ balance is also intimately related to renal control of acid-base balance. The K^+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended¹⁸. Further, active phyto-constituents like alkaloids, glycosides, flavonoids, terpenoids and steroids are known to be responsible for diuretic activities^{19, 20, 21}. These are found to be present in these plant species.

These experimental results have established a pharmacological evidence for the folklore claim about the usefulness of extract *S. pinnata* (Linn.F) Kurz stem bark. Further, to study the possible mechanism of actions and isolation of active principles responsible for such activity.

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Table 1. Phyto-chemical screening for *Spondias pinnata* stem bark extracts

Sl. No.	Constituents and their respective test	Extracts	
		Acetone	Ethanol
1.	Carbohydrates		
2.	Glycosides	-	+
3.	Saponins	+	+
4.	Flavonoids	-	+
5.	Phytosterols	+	+
6.	Phenolic compounds and tannins	+	+
7.	Tri-terpenoids	+	+

(+): Present, (-): Absent

Table 2: Diuretic activity of different extracts of *Spondias pinnata*

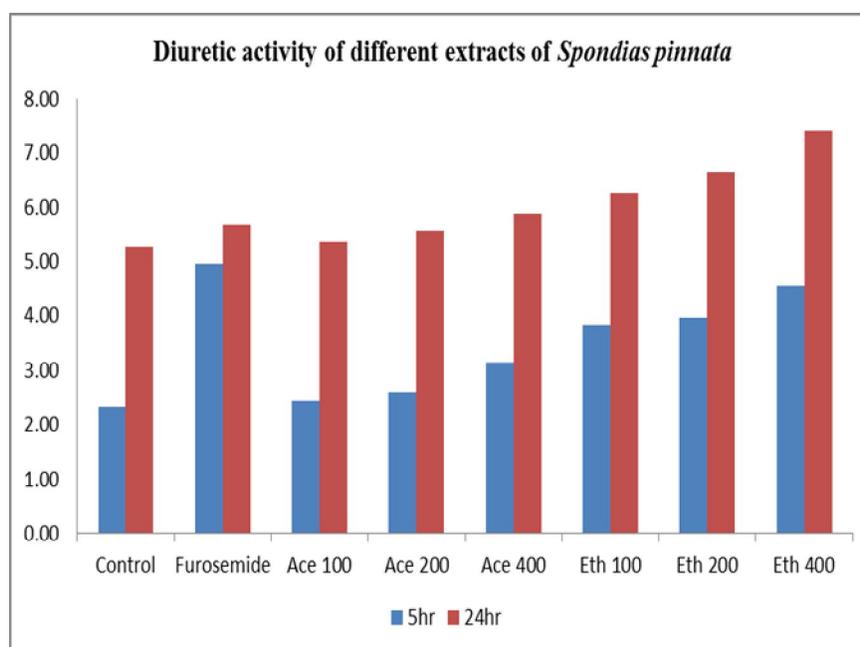
Treatment Groups (mg/kg.p.o)	Volume of urine in ml		Concentration of ions (meq./l) at 24 hr			Na ⁺ /K ⁺
	5hr	24hr	Na ⁺	K ⁺	Cl ⁻	
Vehicle (10ml/kg)	2.34±0.23	5.25±0.17	83.69±1.30	64.02±0.99	88.85±1.32	1.31
Furosemide 10mg/kg	4.96±0.32***	7.68±0.24***	126.03±1.66***	91.70±1.29***	102.33±2.09***	1.37
ACE 100	2.45±0.20	5.38±0.20	85.86±0.98	64.53±1.91	89.20±1.17	1.33
ACE 200	2.59±0.16	5.57±0.22	88.53±0.79	65.20±1.11	90.86±1.23	1.36
ACE 400	3.15±0.26*	5.88±0.15	94.86±1.33*	70.36±1.57*	92.20±0.98	1.35
ETH 100	3.83±0.14***	6.26±0.17*	98.53±2.41**	77.53±1.37**	93.03±1.22	1.27
ETH 200	#3.97±0.19***	#6.65±0.31***	101.33±1.94***	76.53±1.72***	93.53±1.21	1.32
ETH 400	#4.55±0.22***	#7.40±0.21***	#118.53±0.79***	#84.20±2.05***	#95.86±0.63**	1.41

All values are expressed in Mean ± SEM, n=6

* There is significant difference between control and other groups at *** P < 0.001,

** P < 0.01 and P < 0.05 significant level.

There is no significant difference between standard and test drug at #P < 0.05 significant level.

**Fig. 1: Diuretic activity of different extracts of *Spondias pinnata*****REFERENCES**

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