

Research Article

Comparative Study of Anti-Pyretic Activity between Acetone and Ethanol Stem Bark Extracts of *Spondias pinnata* (Linn.F) Kurz.

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ABSTRACT

Spondias pinnata (Linn.F) Kurz (Anacardiaceae) is a medicinal plant and is widely distributed in tropical and subtropical regions of India. The present investigation attempted to find out the anti-pyretic potentials of the acetone and methanol extract of *Spondias pinnata* (Linn.F) Kurz stem bark. The extract was evaluated for phytochemical screening, which indicated the presence of carbohydrate, glycoside, saponin, flavonoids, phytosterol, phenolic compound, tannins and tri-terpenoids. The anti-pyretic activity was evaluated by Brewer's yeast induced pyrexia in albino rats. The ethanol extract at the dose of 200 and 400 mg/kg p.o., showed the significant reduction in yeast induced elevated temperature in a dose depended manner and the effect also extended up to 5 hours after the drug administration. The results of this study indicated that the ethanol extract from stem bark of *S. pinnata* (Linn.F) Kurz possess significant anti-pyretic activities in rodent models.

Key words: Anti-pyretic; *Spondias pinnata* (Linn.F) Kurz; Acetone extract; Ethanolic extract; Yeast.

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INTRODUCTION

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 activity [1-6]. 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 3 [7].

The plant is reported to contain β -Amyrin and oleanolic acid, glycine, cystine, serine, alanine and leucine in fruits. Lignoseric acid, β -sitosterol and its glucoside in aerial parts [8].

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

There are no reports on systematic and scientific study of anti-pyretic activity of bark extracts. On

the basis of the traditional use of the plant as an anti-pyretic agent, we have evaluated the acetone and ethanol stem bark extract for possible anti-pyretic activity in an experimental albino rat model to substantiate the folklore claim. The effect of acetone and ethanol stem bark extract was also compared with that of standard drug Paracetamol, a well-known anti-pyretic agent.

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 and preliminary phytochemical screening

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.

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 indicate the presence of carbohydrate, glycoside, saponin, flavonoids, phenolic compounds, phytosterols and tri-terpenoids.

Animals

Wister Albino rats of both sex weighing (150-200) gram were used for the evaluation of antipyretic 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 hr [12].

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

Animal grouping for tests

Anti-pyretic activities were determined in albino rats of either sex. Rats were divided into 8 groups of 6 animals in each group:

Group 1 Untreated control (0.5% Tween 80 in distilled water and received the dose of 10 ml/kg body weight), Group 2 Standard (received Paracetamol 100mg/kg body weight), Group 3 to Group 5 received acetone extract of stem bark and Group 6 to Group 8 received ethanol extract of stem bark respectively at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight (p.o).

Study on normal body temperature of rats

Animals were divided into eight groups comprising six rats in each group. Only healthy rats with constant normal body temperature were selected for this experiment. The body temperature of each rat was measured rectally recorded again with 1 hr interval up to 5 hr. The maximum reduction in rectal temperature in comparison to the control group was calculated. The results were compared with the effect of standard drug, i.e. Paracetamol 100 mg/kg p.o.

Evaluation of anti-pyretic activity in yeast induced elevated temperature

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in rats [13,14]. Rats were divided into 8 groups of 6 animals in each group. For the measurement of initial body temperature of each rat, a thermistor probe was inserted 3-4 cm. in to the rectum and fastened to the tail by adhesive tape. Temperatures were recorded by a

thermometer at predetermined time intervals after the administration of yeast extract, standard and test drug orally.

As the rats grouped earlier, fever was induced in each rat by the method described. Each rat was given a subcutaneous injection of 10 ml/kg of 15 % w/v Brewer's yeast extract suspension below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food was withdrawn, 18 hr post challenge, the rise in rectal temperature was recorded. The measurement was repeated after 60 min. only animals with body temperature of at least 38°C were taken into the test. At 18th hour following yeast injection, Group 1 served as solvent control 0.5% Tween 80 in distilled water and received the dose of 10 ml/kg body weight, the 2nd group of animals received the standard drug, paracetamol at a dose of 100 mg/kg body weight p.o. and Group 3 to Group 5 received acetone extract of stem bark and Group 6 to Group 8 received ethanol extract of stem bark respectively at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight (p.o). Then, the rats were restrained for recording of their rectal temperature at one hour's interval up to the 5th hour.

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

The effect of yeast induced pyrexia and the anti-pyretic action of acetone and ethanol extract of *S. Pinnata* (Linn.F) Kurz stem bark in rats were shown in Table 2. The result showed that the subcutaneous injection of yeast extract markedly elevated the rectal temperature after 0 hours of administration. The standard drug paracetamol at a dose of 100 mg/kg body weight p.o. significantly reduced the yeast-provoked elevation of body temperature. Treatment with the ethanol extract of *S. pinnata* (Linn.F) Kurz stem bark at the doses of 200 mg/kg body weight showed there was no significance difference with standard drug paracetamol ($P < 0.05$) at 2 hr onwards and 400 mg/kg showed ($P < 0.05$) at 1 hr onwards. The result showed antipyretic activity throughout the observation period up to 5 hours.

The anti-pyretic activities of many plants have been attributed to their saponin, terpenoids, flavonoids, alkaloids and steroids contents [15, 16]. The anti-pyretic activities exhibited by this extract compared with the standard drug paracetamol respectively.

It is well known that paracetamol is a good and promptly acting antipyretic and is a poor inhibitor of prostaglandins synthesis in peripheral tissues, but more active on cyclooxygenase in brain [17]. The ethanol extract of *S. pinnata* (Linn.F) Kurz stem bark produced significant antipyretic activity in Brewer's yeast induced pyrexia in rats.

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

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

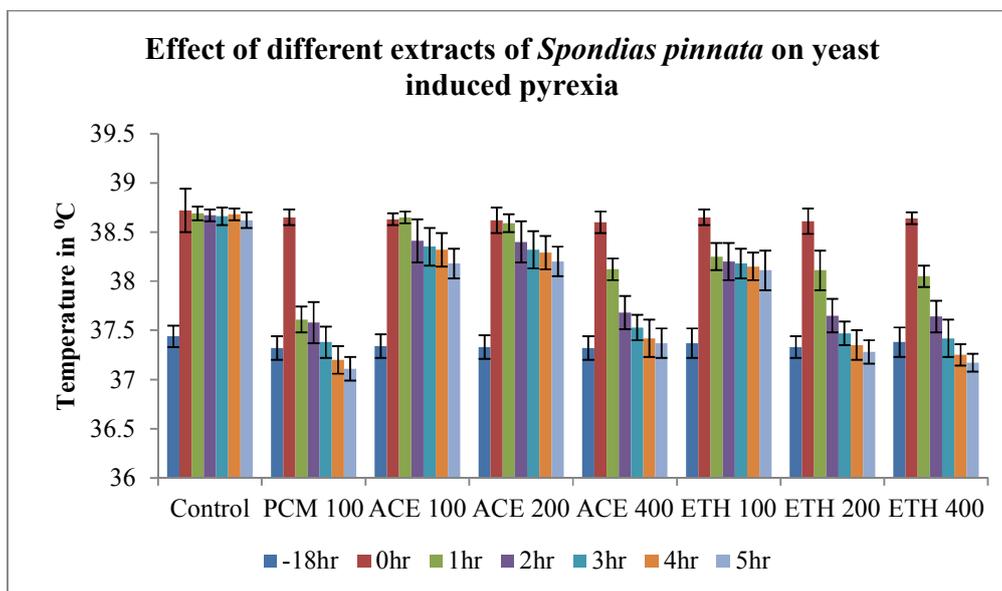
Table 2. Antipyretic activity of *Spondias pinnata* extracts on yeast induced pyrexia.

Groups (mg/kg)	Rectal temperature ($^{\circ}$ C) after yeast injection						
	-18hr	0hr	1hr	2hr	3hr	4hr	5hr
Control 10ml/kg	37.44 \pm 0.11	38.72 \pm 0.22	38.69 \pm 0.07	38.67 \pm 0.06	38.66 \pm 0.09	38.68 \pm 0.06	38.62 \pm 0.08
PCM 100	37.32 \pm 0.12	38.65 \pm 0.08	37.61 \pm 0.13***	37.58 \pm 0.21***	37.38 \pm 0.16***	37.20 \pm 0.14***	37.11 \pm 0.12***
ACE 100	37.34 \pm 0.12	38.63 \pm 0.06	38.65 \pm 0.06	38.41 \pm 0.22	38.35 \pm 0.19	38.32 \pm 0.17	38.18 \pm 0.15
ACE 200	37.33 \pm 0.12	38.62 \pm 0.13	38.59 \pm 0.09	38.40 \pm 0.21	38.32 \pm 0.19	38.29 \pm 0.17	38.20 \pm 0.15
ACE 400	37.32 \pm 0.12	38.60 \pm 0.11	38.12 \pm 0.11*	37.68 \pm 0.17***	37.53 \pm 0.13***	37.42 \pm 0.19***	37.37 \pm 0.15***
ETH 100	37.37 \pm 0.15	38.65 \pm 0.08	38.25 \pm 0.14	38.20 \pm 0.19	38.18 \pm 0.15	38.15 \pm 0.14	38.11 \pm 0.20
ETH 200	37.33 \pm 0.11	38.61 \pm 0.13	38.11 \pm 0.20*	#37.65 \pm 0.17***	#37.47 \pm 0.12***	#37.35 \pm 0.15***	#37.28 \pm 0.12***
ETH 400	37.38 \pm 0.15	38.64 \pm 0.06	#38.05 \pm 0.11*	#37.64 \pm 0.16***	#37.42 \pm 0.19***	#37.25 \pm 0.11***	#37.17 \pm 0.09***

All values are expressed in Mean \pm 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 Effect of different extracts of *Spondias pinnata* on yeast induced pyrexia

CONCLUSION

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 principle(s) responsible for such activity.

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